CENTER for PRODUCE SAFETY

**CPS 2013 RFP**
**FINAL PROJECT REPORT**

## Project Title
Enhancement of forced-air cooling to reduce Listeria monocytogenes, Salmonella, and/or total surface microbiota on cantaloupes

## Project Period
January 1, 2014 – December 31, 2014

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## Objectives
*1. To identify optimal treatment conditions to reduce L. monocytogenes, Salmonella and/or spoilage microbiota contamination of cantaloupes during the cooling step using an experimental system.*

*2. To assess the effects of optimal treatment conditions on the reduction of natural spoilage microorganisms, as well as determine the degree of any changes to the quality and sensory properties of cantaloupes after cooling in our experimental system.*

## Funding for this project provided by the Center for Produce Safety through:
CPS Campaign for Research

## FINAL REPORT

## Abstract

Introduction: Cantaloupes have been associated with several serious foodborne disease outbreaks in the US. Forced air cooling is a most widely adopted cooling method for produce commodities, including cantaloupes. This process can provide a unique opportunity for antimicrobial intervention to reduce foodborne pathogens and spoilage organisms contaminating the surface of harvested cantaloupes while maintaining its quality. It is generally acknowledged that aqueous wash-based systems have a limited ability to effectively eliminate foodborne pathogens. Ultrasonic vaporizers (or atomizers) can be used in atomization of liquid sanitizers, and thus generate aerosolized sanitizers. Aerosolized sanitizers diffused like gaseous sanitizers and that diffusion of aerosolized sanitizers in a chamber was not affected by height or orientation. Thus, aerosolization with its high penetration ability and broad spectrum of applicable sanitizers, has the potential to be an alternative sanitizer delivery system in the forced air cooling process.

Purpose: Limited studies have investigated the effectiveness of aerosolized sanitizers against pathogens on fresh cantaloupes during the forced air cooling process. In our funded Proof of Concept research, we developed a sanitizing process using novel combination of ultrasonic atomization technology and antimicrobial formulations which can be integrated into commercial forced-air cooling systems. We focused on evaluation of this approach to reduce *L. monocytogenes*, *Salmonella* and/or spoilage organisms during cooling of cantaloupes placed inside Regular cartons, without affecting final quality and sensory attributes.

Methods: Ultrasonic vaporizers were integrated into an experimental forced-air cooling system to study the potential enhancement of cantaloupe safety and quality. Various operation conditions, including temperature and treatment duration, were investigated to optimize the process with assistance from our industry collaborator. Antimicrobial formulations were developed to take advantages of synergism of different antimicrobials to inactivate foodborne pathogens on the surface of cantaloupes. Aqueous antimicrobials were aerosolized by ultrasonic atomization and delivered into the experimental system. The efficacy of aerosolized antimicrobials were evaluated by reductions of *Listeria monocytogenes, Salmonella* and/or spoilage microbiota on the cantaloupe placed inside a Regular cartons during the forced-air cooling process. The impacts of selected effective treatments on cantaloupe quality were also evaluated in day 0, 1, 5, 10 at 4 °C for changes of color, texture, pH, total phenolics, soluble solids content, total aerobic bacterial plate counts, and yeast and mold counts during the storage.

Results: Treatment duration had varied effects on pathogen inactivation depending on the antimicrobial formulations. Antimicrobial formulations including both antimicrobial compositions and concentrations played a major role on the inactivation of pathogens inoculated on cantaloupe surface placed inside a Regular carton. Formulation with thyme oil, citric acid and SDS demonstrated biggest potential to inactivate both pathogens, with 0.9 ± 0.4 log CFU/g reduction on *Salmonella*, 0.7± 0.5 log CFU/g reduction on *L. monocytogenes* respectively. Formulation with chlorinated solution and SDS also had interesting inactivation efficacy but with bigger variations. The aerosolized antimicrobials were able to pass through the opening holes of a carton to inactivate foodborne pathogens or spoilage organisms during 180 min treatment. Higher inactivation efficacy was generally observed on grape tomatoes when compared with findings on cantaloupes. The approaches had little or minimal risk of cross-contamination.

Treatment with aerosolized chlorinated/SDS solution during 180 min forced air cooling of cantaloupes had no negative quality changes on the cantaloupes subsequently stored at 4 □C for 10 days.

Significance: This study provided important data on the feasibility of using the proposed approach to reduce *L. monocytogenes*, *Salmonella* and/or spoilage organisms during cooling of cantaloupes without affecting final quality and sensory attributes.

## Background

Forced air cooling is a most widely adopted cooling method for produce commodities, including cantaloupes which have been implicated in several costly foodborne illness outbreaks (CDC, 1991, 2002, 2012; Bowen et al., 2006). This process can provide a unique opportunity for antimicrobial intervention to reduce or even eliminate foodborne pathogens and spoilage organisms contaminating the surface of harvested cantaloupes while maintaining its quality.

It is generally acknowledged that aqueous wash-based systems have a limited ability to effectively eliminate foodborne pathogens (Alvarado-Casillas et al., 2007). A gaseous sanitation system is expected to be more effective because of the faster diffusion of sanitizer molecules in a gas than a liquid phase. Because forced air cooling involves moving cold air over the product by using a pressure gradient to force the air through the product container, gaseous sanitizers can feasibly be integrated into such cooling systems. Among the commonly used or studied sanitizers, only chlorine dioxide, chlorine and ozone are gaseous, but the environmental risks and higher requirements for gas usage limit their applications within current cooling systems. In our proposed project, we will take advantage of our experience with a novel combination of ultrasonic atomization technology and an antimicrobial formulation to effectively apply sanitizers during the forced air cooling process.

Ultrasonic vaporizers (or atomizers) can be used in atomization of liquid sanitizers, and thus generate aerosolized sanitizers. Aerosolization is defined as the dispersion of liquid as a fine mist in air. Oh et al. (2005) reported that aerosolized sanitizers diffused like gaseous sanitizers and that diffusion of aerosolized sanitizers in a chamber was not affected by height or orientation, which is not the case for a spraying system. The same group also assessed the efficacy of aerosolized peroxyacetic acid as a produce sanitizer. *E. coli* O157:H7, *S.* Typhimurium and *L. monocytogenes* were reduced by 3.4, 4.5 and 3.8 log CFU/g, respectively after a 60-min treatment. Also, a 2-min treatment with ultrasonically atomized 5% allyl isothiocyanate reduced *E. coli* O157:H7 on spinach leaves by 3.3 log CFU/g after 10-day storage at 4 °C without causing noticeable adverse effects on the appearance of the samples (Huang 2011). Aerosolized lactic acid (LA) has also been used to disinfect chicken houses, and Fiser (1978) reported that continuous disinfection by aerosolized LA resulted in an improved state of health of chickens. Thus, aerosolization with its high penetration ability and broad spectrum of applicable sanitizers, has the potential to be an alternative sanitizer delivery system. However, limited studies have investigated the effectiveness of aerosolized sanitizers against pathogens on fresh produce during the forced air cooling process. In our funded Proof of Concept research, ultrasonic vaporizers were integrated into an experimental forced-air cooling system for potential enhancement of cantaloupe safety and quality.

## Research Methods and Results

**Research methods to determine the impacts of atomization of antimicrobial solution to reduce *L. monocytogenes, Salmonella* and/or spoilage microbiota contamination on cantaloupes.**

**1. Bacterial strains and inoculum preparation.** Wild-type strains including *Salmonella* (*S.* St. Paul 02-517-1 cantaloupe outbreak isolate) and *L. monocytogenes* (390-1 cantaloupe outbreak isolate) were used in our study. Wild strains were obtained from culture collection in Department of Animal and Food Sciences at University of Delaware. The *Salmonella* strain was adapted to grow in the presence of nalidixic acid (50 µg/mL, Fisher Scientific, Hampton, NH, USA) alone to create a single antibiotic resistance strain. *Salmonella* strain was grown on tryptic soy agar (TSA, Difco Laboratories, Sparks, MD, USA) supplemented with nalidixic acid (TSA-N) for 2-3 days at 35 ℃. *Listeria* strain was grown on TSA plates without any supplement. Single colonies were picked and transferred to 10 mL of tryptic soy broth (TSB, Difco) supplemented with same antibiotic (TSB-N) for *Salmonella* and 10mL TSB for *Listeria*. The culture was incubated at 35 ℃ overnight and second-transferred to 10 mL of fresh TSB-N or TSB to yield an approximate population of $10^9$ CFU/mL after 24 h incubation at 35 ℃. The culture was diluted to $10^8$ CFU/mL using sterile 0.1 % peptone water (Difco) and used as inoculum.

**2. Cantaloupe preparation and inoculation.** Fresh cantaloupe were purchased from a local supermarket and stored at 4±2 °C for a maximum of 4 h before use. To facilitate the screening process in the project, rind was removed and cut into small size (~1 g) using a sterilized knife. To spot inoculate cantaloupe rind pieces, 10 µL of $10^8$ CFU/mL inoculum was deposited on the outside surface of each rind piece as one droplet. Inoculated cantaloupe rind pieces were dried in the biosafety hood for 2h before use.

**3 Inactivation of *Salmonella* and *Listeria* using an experimental system.** Inoculated cantaloupe rind pieces were transferred to biosafety level 2 laboratory in sterile 6-well culture plates and placed in our experimental system (a forced air cooling chamber added with an ultrasonic mister). Two “Regular” cartons (16 11/16 length X 12 ¾ width X 9 7/8 depth) were stacked up inside the chamber. Cantaloupe rind pieces without inoculation were placed in the bottom carton to evaluate potential cross-contamination. Inoculated pieces were placed inside the top carton as well as on the top edge of the top carton to simulate two exposure scenarios, inside carton and outside carton. Various antimicrobial solutions with different chemical composition and concentrations (8L) were placed in the bucket; mist was generated by an ultrasonic mister placed inside the bucket, and transported into chamber to fill the whole unit. Treatment duration (60 -360 min) was evaluated at 4 ± 2 °C. The commonly used cantaloupe target cooling temperature (35 - 40 °F), and cooling time (3-4 h) were investigated based on commercial operation parameters suggested by California Melon Research Board. After treatments, cantaloupe rind pieces were taken out for the microbial analysis. Rind pieces without treatment served as positive control. Rind pieces without inoculation or treatment served as negative control to determine potential background *Salmonella* and *Listeria* levels.

**4. Microbial analyses.** Cantaloupe rind pieces were microbiologically analyzed at the end treatments. They were transferred into sterile stomacher bags with D/E neutralized broth (Difco) at 1:9 ratio (w:v) and homogenized by stomacher (400 Circulator, Seward Co., West Sussex, UK) at 300 rpm for 2 min to help releasing and evenly distributing pathogens. The homogenate was serially diluted using 0.1% peptone water and plated on TSA-N (for *Salmonella*) or *Listeria* media (*Listeria* selective agar base supplemented with Brilliance$^{TM}$ Listeria differential supplement and Brilliance$^{TM}$ Listeria selective supplement) plates using sterile spreaders. Colonies were enumerated after incubation for 48 h at 37℃. Pathogen survivor population was reported as log 10 CFU/g, and pathogen reductions were calculated as the net difference of

pathogen survivors in treatments and positive controls. When evaluating media impacts on *Salmonella* reduction, a selective media, xylose lysine deoxycholate agar supplemented with nalidixic acid (XLD-N) was included in the experiments for *Salmonella* enumeration.

**Research methods to evaluate the quality and sensory properties of cantaloupes after cooling in our experimental system**

**1. Cantaloupe preparation.** Fresh cantaloupes were purchased from a local supermarket and stored at 4 ± 2 °C for a maximum of 4 h before use. Medium size cantaloupes with appealing firmness were selected. The selected cantaloupes were intact and had no noticeable physical injury. Whole cantaloupes without inoculation were used for quality analysis.

**2. Treatments.** Cantaloupes were placed inside the forced air chamber. Two cartons were stacked up in the chamber. All cantaloupes were placed inside the top cartons. Effective antimicrobial solutions at 8 L were placed in the bucket. Mist was generated by an ultrasonic mister placed inside the bucket and transported into chamber to fill the whole unit. After 3 hours of treatment at 4 ± 2 °C, cantaloupes were removed for quality analysis stored in a refrigerator at 4 ± 1 ℃ for 10 days. Cantaloupes without treatment served as control. Whole cantaloupes were randomly selected at sampling day and fresh-cut for the quality analysis.

**3. Effect of our treatments on color, texture, total soluble solids, pH and titratable acidity of cantaloupe flesh.** The color and texture of cantaloupe flesh were analyzed at day 0 (right after treatment), day 5 and day 10. Color was tested by using chromameter (Minolta CR-10, Minolta, Osaka, Japan). Color parameters will be quantified in the Hunter L* (lightness/darkness), a* (redness/greenness), and b* (yellowness/blueness). Texture of cantaloupe flesh was measured as shear strength using a texture analyzer (TA. XT2i, Texture Technologies, New York, USA). Samples were placed on the TA-91 platform and compression tests were performed. The maximum force (Fmax) needed to compress the samples were reported. Total soluble solid of the cantaloupe juice was tested by using portable refractometer, and pH of the cantaloupe juice was measured by pH meter (Oakton Waterproof pH Testr® 20, Vernon Hills, IL USA).

**4. Effect of treatments on TPC of cantaloupe flesh**. TPC was determined as described by Lu et al. (2010) with slight modification. Cantaloupe flesh pieces were homogenized in stomacher bags with 25 mL of ethanol with the ratio of 1:5 (w/v). The bags were heat sealed and the polyphenol compounds was extracted by ethanol with gentle massaging for 30 min. Extractions were centrifuged at 4000 rpm for 10min (Beckman Coulter, Inc. Microfuge®, Indianapolis, IN, U.S.) and supernatants were collected. The 30 µL of supernatants (dilute if necessary) was added into clear 96-well plate with a clear bottom followed by adding 60 µL of the Folin–Ciocalteau reagent ($1\times10^{-4}$ M) and 120 µL of sodium carbonate solution (20%, w:v). The plate was covered with clear lid, shaken horizontally for full mixture and incubated for 30 min at 35℃. A serial of known concentration of gallic acid was used as standards. Absorbance was measured at 765 nm using the Synergy $^{TM\,2}$ multi-mode microplate reader (BioTek Instruments, Inc. Winooski, VT, U.S.). The results were expressed as micrograms of gallic acid equivalents (GAEs) per 100 grams of fresh weight (FW).

**5. Effect of treatments on total bacteria count (TBC) and total yeast and mold count (TYMC) of cantaloupe rind surface.** TBC and TYMC were measured by 3M Petrifilm$^{TM}$ (St. Paul, MN, USA) aerobic count plate and yeast and mold count plate, respectively, according to the manufacture instructions. Survivor population was reported as log 10 CFU/g.

**Statistical analysis**. All microbial experiments were replicated in at least 2 independent trials with 3 repeats in each trial. Results were reported as Mean ± SD. JMP (SAS Cary, NC, USA) software was used for statistical analyses. For inactivation efficacy, results were reported as *Salmonella* or *L.monocytogenes* log reduction (log 10 CFU/g) and student's t test was used to determine difference between each treatment. For shelf-life quality of cantaloupes, student's t test was used to determine difference between treated and control samples on day 0, day 1, day 5, and day 10. Significant difference was reported when $P<0.05$.

## Results

### 1. Efficacy of treatments for reduction of *Salmonella* and *L.monocytogenes* contamination during cooling of cantaloupes in our experimental system

As planned in objective 1 in project, we have done extensive studies to identify the optimal treatments for reduction of *Salmonella* and *L.monocytogenes* contamination during cooling of cantaloupes in our experimental system inside a biosafety level-2 room. To minimize the interference from background microflora, nalidixic acid resistant *Salmonella* St. Paul 02-517-1 was prepared in-house and used in all the experiments. *L. monocytogenes* 390-1, was obtained from Dr. Kali Kniel's lab, and used in the experiments. Two controls, an uninoculated and untreated control, and an inoculated and untreated control, were used in all experiments. Various antimicrobial formulations at different concentrations have been tested, as shown in Figs 1, 2, 3, 4 and 5. Treatment durations ranged from 60 min to 360 min were evaluated to determine its impacts on pathogen reduction (Fig 1). The impacts of surface structures were determined using grape tomatoes and cantaloupes inside the same experimental system during the same treatments (Figs 2 and 3). Two different media were compared to determine the effects of media materials on *Salmonella* enumeration, which included tryptic soy agar supplemented with nalidixic acid (TSA-N), and xylose lysine deoxycholate agar supplemented with nalidixic acid (XLD-N) (Fig 3). Further experiments were also designed to determine if the exposure location, that is, inside or outside a "Regular" type of carton, affected the pathogen inactivation (Fig 2, 4 and 5). Inactivation of both *Salmonella* and *L.monocytogenes* were determined in our experimental system (Fig 4). The effects of one treatment on both *Salmonella* and *L.monocytogenes* inactivation were compared among three independent trials (Fig 5).

#### *Effects of treatment duration, antimicrobial formulation and exposure level on Salmonella reduction*

Based on our current findings (Fig 1), treatment duration had varied effects to enhance the pathogen inactivation depending on the antimicrobial formulations. For example, treatment Chs (aerosolized chlorinated solution at 200 ppm +100 ppm SDS) for 90 min and 180 min had similar *Salmonella* reductions, 0.7± 0.4 and 0.8 ± 1.0 log CFU/g reduction respectively. While the 270 min Chs treatment had bigger *Salmonella* (1.2 ± 0.0 log CFU/g) reduction, increase of treatment duration to 360 min did not further enhance the efficacy. The longer treatment duration actually decreased the efficacy, however, the condensation of aerosols was also observed in the experiments with duration longer than 180 min due to limited power of forced air fan used in our experimental cooling system. For other antimicrobial formulations with minimal decontamination efficacy, treatment duration had little effects on the pathogen log reduction. Considering the limitation of our system and real application, treatment duration at 180 min was used in all subsequent experiments.

Antimicrobial formulations including both antimicrobial compositions and concentrations played a major role on the inactivation efficacy. The highest concentrations of antimicrobials were used in treatment Chs, compared with those of other treatments in Fig 1, which might help to explain highest efficacy in treatment Chs. Surprisingly, treatment PSC (aerosolized solution of 80 ppm peroxyacetic acid +100ppm SDS +100ppm *trans*-cinnamaldehyde) did not have comparable efficacy as observed in Chs treatment, which could be due to the limited comparability or stabilities of antimicrobial components in this aerosolized formulation. Further research was done to determine this possibility (Fig 2). After using a commercial peroxyacetic acid products StorOx 2.0 at high (100 ppm) or low (85 ppm) concentrations for 180 min, the efficacy increased (0.4-0.5 log CFU/g reduction) but not substantially. No significant difference was obtained between the two concentrations. Formulation of PHAS (aerosolized solution of 80ppm peroxyacetic acid + 600 ppm hydrogen peroxide+600 ppm acetic acid+100 ppm SDS) did not further enhance the efficacy when compared with StorOx 2.0. ES had similar inactivation as compared with PHAS. To our surprise, no significant difference were determined between *Salmonella* population of cantaloupes placed inside and those outside a “Regular” type of carton (Fig 2). The full exposure to the aerosolized formulations did not enhance the decontamination.

Other findings on the impacts of antimicrobial formulations and surface structures were also summarized in Fig 2. Interestingly, higher inactivation efficacy was generally observed on grape tomatoes when compared with findings on cantaloupes ($p<0.05$), with exception of ES treatment. StorOx 2.0 at high and low concentrations decontaminated grape tomatoes by 1.5 ± 0.0 and 1.0 ± 0.2 log CFU/g reduction, respectively. PHAS inactivated approximately 0.6 ± 0.3 log CFU/g of *Salmonella* on grape tomatoes placed inside the carton. The higher decontamination efficacy could be related with much more smooth surface of tomatoes, compared with the netted cantaloupe surface. In addition, there is no significant difference between *Salmonella* population of grape tomatoes or cantaloupes placed inside and those outside a “Regular” type of cartons, which suggested that aerosolized formulations distributed uniformly inside the entire experimental cooling system and the aerosols were able to pass through the opening holes of a carton. The cooling of the melon produced a negative pressure and might help drive the air together with the aerosolized antimicrobials into the melons.

Shown in Fig 3, higher inactivation efficacy was still observed on grape tomatoes when compared with findings on cantaloupes. The two different media provided different *Salmonella* enumeration, with higher inactivation reported when using XLD-N compared to that using TSA-N. This finding could be due to less growth of injured cells in selective media. However, big variation made it difficult to favor XLD-N, a selective medium not suitable for injured cells. As a result, TSA-N was used for all other *Salmonella* enumeration.

Experiments using grape tomatoes and XLD-N media were conducted to determine whether produce surface structure or enumeration media contributed to large variations, both of which were not initially planned. The large variations observed in some experiments made it difficult to quickly identify inactivation efficacy and more trials became necessary to confirm the findings. In addition, as required by our University Environmental Health & Safety, extensive decontamination between each trial were performed because human pathogens were involved in our experiments. As a result, we modified our approach to simultaneously evaluate the reduction of *L.monocytogenes* and *Salmonella* contamination during cooling of cantaloupes in our experimental system.

***Impacts of treatments on simultaneous reduction of both Salmonella and L. monocytogenes***

The data so far generally showed that the effective treatments resulted in reduction of both pathogens (Fig 4 and 5). Among all the antimicrobial formulation tested (Fig 4), ToCS (4 mg/mL Thyme oil + 2.5% Citric acid+1000 ppm SDS) demonstrated biggest potential to inactivate both pathogens, with 0.9 ± 0.4 log CFU/g reduction on *Salmonella* ($p<0.05$), 0.7± 0.5 log CFU/g reduction on *L. monocytogenes.* This treatment also resulted in TYMC and TBC at 0.5 ± 0.8 and 0.1 ±0.1 log CFU/g reductions, respectively. ToChS (4 mg/mL Thyme oil + 500 ppm Chlorine+1000 ppm SDS) also resulted in TYMC and TBC at 1.2 ± 0.2 and 0.2 ±0.7 log CFU/g reductions, respectively.

For all the five treatments, higher variations were normally determined in *L. monocytogenes* reductions. For example, when treating cantaloupe using aerosolized solution of TcinScar (400 ppm thymol + 400 ppm trans-cinnmaldehyde+400 ppm carvacrol+100 ppm SDS) in 3 different testing days (3 repeats each day), the reduction of *L. monocytogenes* varied between 2.6 ± 2.6 log CFU/g and 0.6 ± 1.0 log CFU/g for cantaloupes placed inside carton among the three independent trials (Fig 5). These large variations could be related to variations in the surface microstructure and differences in the microbiota on the cantaloupes. In a recent research using Cryo-scanning electron microscopy (Chang et al., 2014), cantaloupe samples inoculated with *Salmonella or L. monocytogenes* showed biofilm formation on the surface after 1 and half hour incubation at room temperature. Based on this finding, inoculated cantaloupe rind pieces in our research were dried in the biosafety hood for 2h before treatments and this could result in biofilm formation which made pathogen inactivation more difficult. Previous search did not distinguish the biofilm formation between *Salmonella and L. monocytogenes,* but we hypothesized that *L. monocytogenes* might form biofilm in more irregular pattern and contribute to bigger variation in *L. monocytogenes* reductions. However, more research is need to understand the big variations.

Furthermore, in commercial forced air cooling, one or several large permanently mounted fan delivers the forced air to be circulated through the vented cartons and around the packed fruits. Due to limitation of our system, the flow rate of our forced air fan could be far less comparable with the one used in commercial scale. This could contribute to the limited efficacy when the rougher surface structure was used in one trial compared to the others.

One major concern of this project is to determine if the novel approaches could result in potential risk of cross-contamination. In all the aforementioned experiments, uninoculated cantaloupe samples were put inside another carton underneath the carton containing the inoculated cantaloupe samples during the same aerosolized treatment, and then evaluated for *Salmonella* or *L. monocytogenes* population. No *Salmonella* or *L. monocytogenes* population were observed in these uninoculated samples.

## 2. Effects of treatments on cantaloupe quality during the shelf-life.

After whole cantaloupes treated with Chs or received no treatment, the cantaloupes were evaluated at day 0, 1, 5, 10 at 4 □. Texture, color, pH, total phenolics, soluble solids content, total aerobic bacterial plate counts, and yeast and mold counts. No significant quality changes were observed between the treatment and control during the storage (Table 1 and Picture 1). Texture was decreased over time for both control and treatment, from 9.98 ± 1.29N and 14.21± 5.81N, respectively in day 0, to 5.31 ± 2.04 and 5.72 ± 0.89 respectively in day 10. The findings indicated cantaloupes became softer over time, but no significant difference were determined between control and treatment. Treated cantaloupes did have firmer texture in day 0, 1 and day 5 compared with the values obtained on controls, but we could not distinguish this impact from treatment or from the natural variation in cantaloupe samples as we used different

whole cantaloupe in the sampling days. Similar change pattern was also observed for soluble solids content. No negative impacts were observed on visible sensory properties.

## Outcomes and Accomplishments

There are six major accomplishments during whole project period.
1) Determined the impacts of treatment durations on *Salmonella* inactivation. Treatment duration had varied effects to enhance the pathogen inactivation depending on the antimicrobial formulations. For treatments showing pathogen reduction, increase of treatment duration might result in bigger reduction but it had an optimum duration. Increase of treatment duration beyond this optimum duration did not further enhance the efficacy.
2) Determined the impacts of surface structures on *Salmonella* inactivation. Higher inactivation efficacy was generally observed on grape tomatoes when compared with findings on cantaloupes. The higher decontamination efficacy could be related with much more smooth surface of tomatoes, compared with the netted cantaloupe surface.
3) Determined the impacts of various antimicrobial formulations on *Salmonella* and *L. monocytogenes* inactivation. Antimicrobial formulations including both antimicrobial compositions and concentrations played a major role on the inactivation efficacy. Formulation with thyme oil, citric acid and SDS demonstrated biggest potential to inactivate both pathogens.
4) Determined the impacts of exposure locations on *Salmonella* and *L. monocytogenes* inactivation. There is no significant difference between *Salmonella* or *L. monocytogenes* reductions placed inside and those outside a “Regular” type of carton, which suggested that aerosolized formulations distributed uniformly inside the entire experimental cooling system and the aerosols were able to pass through the opening holes of a carton.
5) Determined the impacts of novel approaches on potential risk of cross-contamination. In all the aforementioned experiments, uninoculated cantaloupe samples were put inside another carton underneath the carton containing the inoculated cantaloupe samples during the same aerosolized treatment, and then evaluated for *Salmonella* or *L. monocytogenes* population. No *Salmonella* or *L. monocytogenes* population were observed in these uninoculated samples.
6) Investigated the impacts of select treatments on cantaloupe quality. Treatment with aerosolized chlorinated solution during 180 min forced air cooling of cantaloupes had no negative quality changes on the cantaloupes stored at 4 □C for 10 days.

In addition, we developed important collaboration with cantaloupe industry and other researchers. With help of CPS, an advisory board was established to assist with our research during the whole research period. From the advisory board, we have obtained cantaloupes and cartons, as well as information on key parameters widely used for forced air cooling of cantaloupe in the industry. All these collaborations helped us to successfully conduct our research. Based on these assistance and our current research findings, we were able to submit a new grant proposal to USDA NIFA program. We are interested to obtain additional funding to translate our current findings from this Proof of Concept project into future applications. We propose in the NIFA proposal to conduct extensive and novel research to understand the big variations of *Salmonella* or *L. monocytogenes* reductions in some treatments. We also propose to identify more efficient treatments and validate the treatment efficacy in a larger scale and on produce with different surface structures.

## Summary of Findings and Recommendations

With a novel combination of ultrasonic atomization and antimicrobial formulation, our approaches could significantly reduce *Salmonella* or *L. monocytogenes* contaminating the surface of cantaloupes placed inside a Regular carton during their forced air cooling process.

The aerosolized antimicrobials were able to pass through the opening holes of a carton to inactivate foodborne pathogens or spoilage organisms. Higher inactivation efficacy was generally observed on grape tomatoes when compared with findings on cantaloupes. The approaches had little or minimal risk of cross-contamination. This process can provide a unique opportunity for antimicrobial intervention to reduce foodborne pathogens and spoilage organisms contaminating the surface of harvested produce while maintaining its quality. More extensive and novel research are still needed to understand better the big variations of *Salmonella* or *L. monocytogenes* reductions in some treatments, and validate the treatment efficacy in a larger scale.

## APPENDICES

### Publications and Presentations (required)

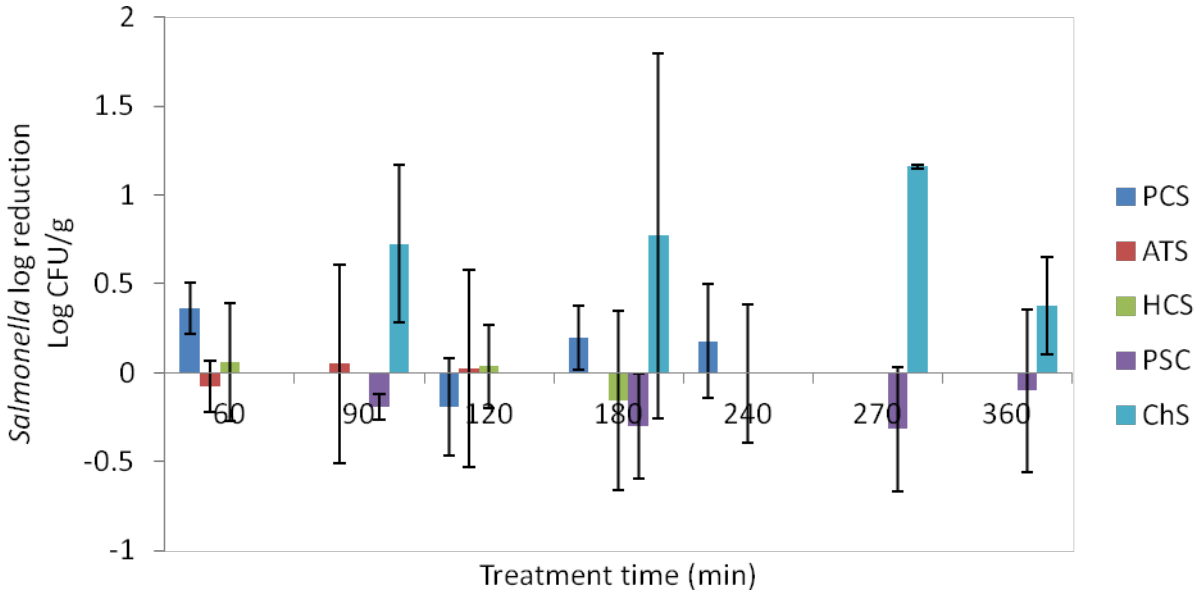
One poster was presented in CPS research symposium in June 2014, and we will present another poster in the coming CPS research symposium in June 2015. We will submit our research finding for publication and other presentations after obtaining the permission of CPS.

### Budget Summary (required)

The allocated budget was fully utilized to execute this project. One fulltime graduate student was supported by the fund to conduct the proposed experiments. Partial tuition support at $8,000 has been provided by the funding for the graduate student. Most of other fund was used to purchase the lab materials or supplies for the research, except the allowed fund for IDC and travel to CPS symposium this June. We are very appreciative of the timely and generous funding that was provided to test our novel approaches.

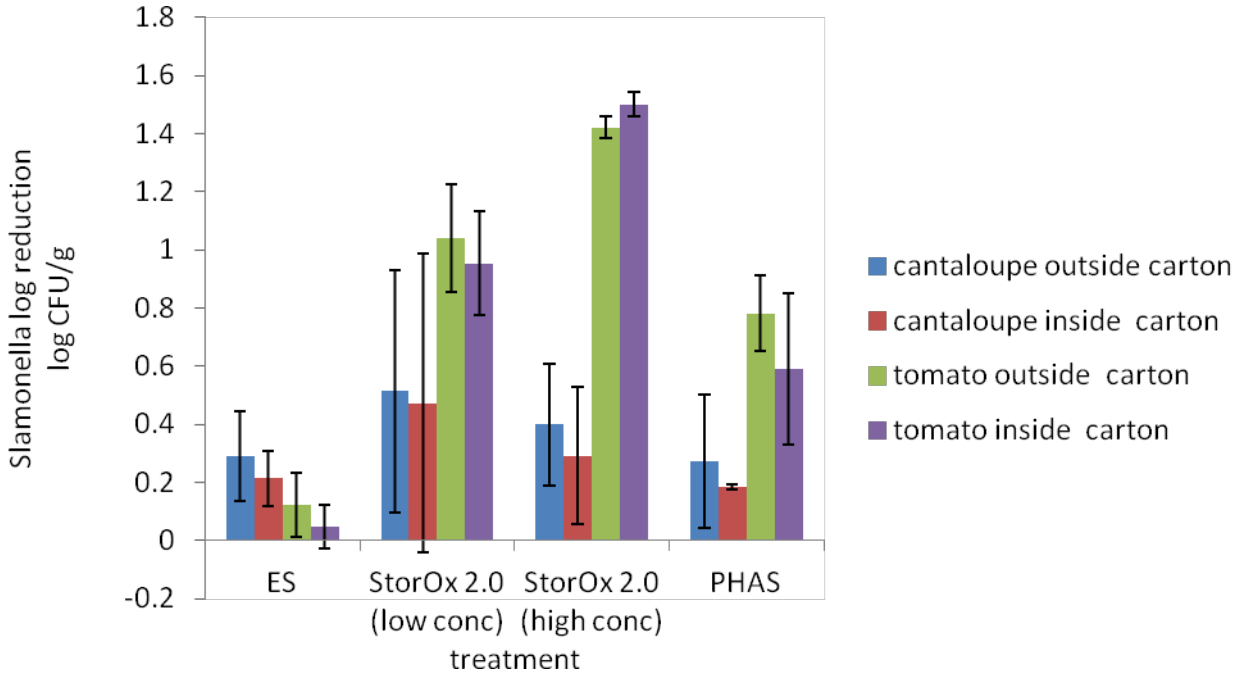
**Tables and Figures (optional)**

Fig 1 The impacts of antimicrobial formulations and treatment duration on *Salmonella* inactivation during forced air cooling of cantaloupes. (Data are average and standard deviation of two independent trials, treatments were conducted inside cartons)



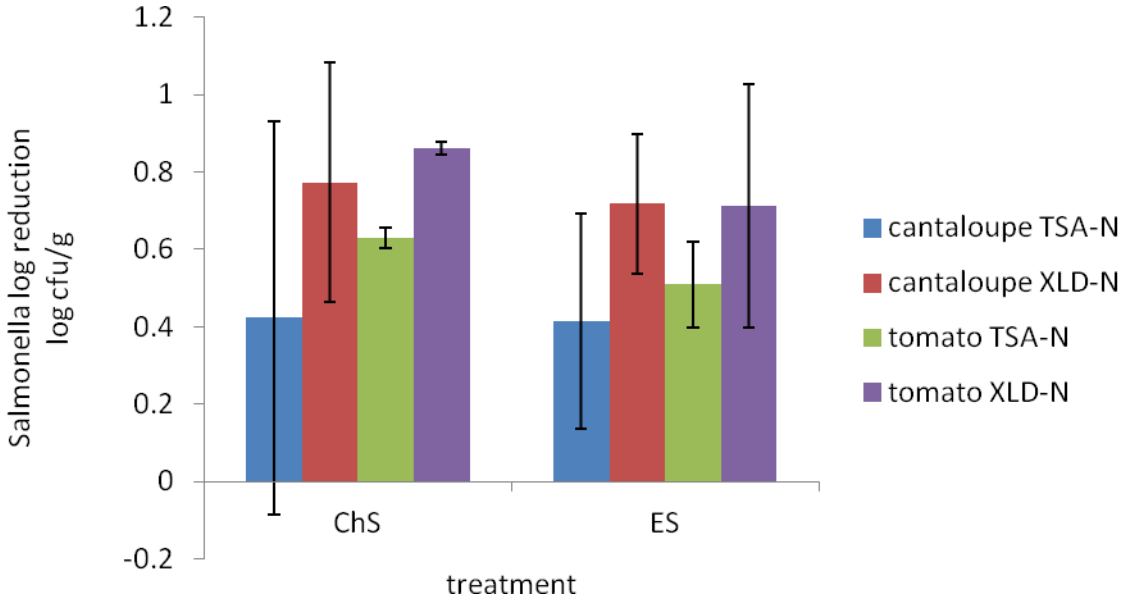

**Note**: PCS, 1 ppm peroxyacetic acid +1ppm *trans*-cinnamaldehyde+ 1ppm SDS;
ATS, 1 ppm acetic acid +1ppm thymol + 1ppm SDS;
HCS, 1 ppm hydrogen peroxide +1ppm *trans*-cinnamaldehyde+ 1ppm SDS;
PSC, 80 ppm peroxyacetic acid +100ppm SDS +100ppm *trans*-cinnamaldehyde;
Chs, chlorinated solution at 200ppm +100ppm SDS, pH adjusted to 6.5.

Fig 2 The impacts of antimicrobial formulations, surface structures and exposure locations on *Salmonella* inactivation during forced air cooling of cantaloupes or grape tomatoes. (Data are average and standard deviation of two independent trials, treatment duration 180 min)



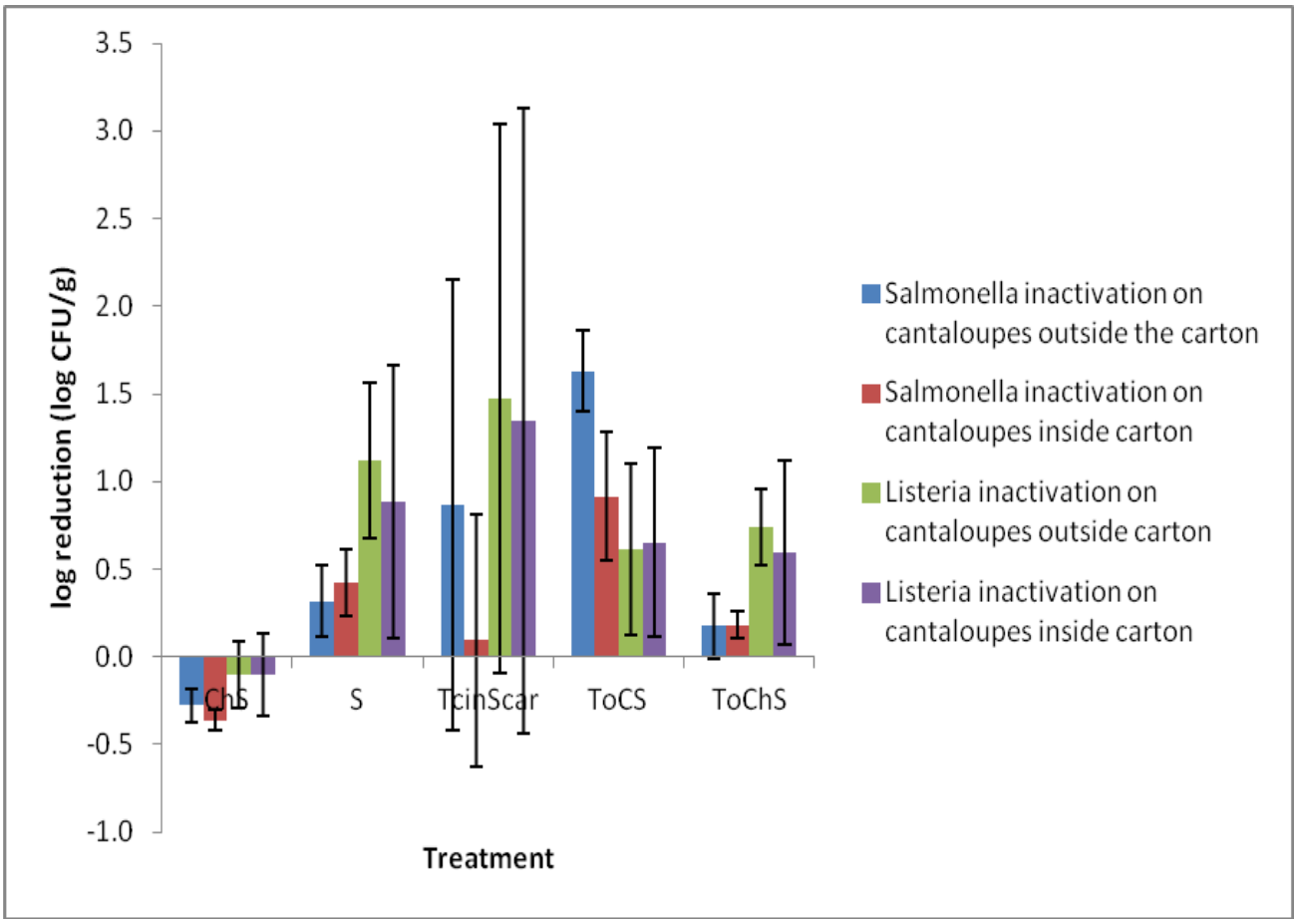

**Note**: ES, 400ppm EP+100ppm SDS;
StorOx 2.0 (low concentration), commercial peroxyacetic acid products StorOx 2.0 at 85 ppm;
StorOx 2.0 (high concentration), commercial peroxyacetic acid products StorOx 2.0 at 100 ppm;
PHAS, 80ppm peroxyacetic acid + 600 ppm hydrogen peroxide+600 ppm acetic acid+100 ppm SDS

Fig 3 The effects of enumeration media materials, antimicrobial formulations, and surface structures on *Salmonella* inactivation during forced air cooling of cantaloupes or grape tomatoes.




**Note**: ES, 400ppm EP+100ppm SDS; Chs, chlorinated solution at 200 ppm +100 ppm SD

Fig 4 The impacts of antimicrobial formulations and exposure locations on *Salmonella* inactivation during forced air cooling of cantaloupes. (Data are average and standard deviation of or 3 independent trials, treatment duration 180 min)




**Note**: ; Chs, chlorinated solution at 200 ppm +100 ppm SDS, pH adjusted to pH=6.5;
S, 100 ppm SDS;
TcinScar, 400 ppm thymol+ 400 ppm trans-cinnmaldehyde+100 ppm SDS+400ppm carcacrol;
ToCS, 4 mg/mL thyme oil +2.5% citric acid+1000 ppm SDS;
ToChS, 4 mg/mL thyme oil + 500 ppm chlorine+1000 ppm SDS

Fig 5 The effects of the same treatment (TcinScar, 400 ppm thymol + 400 ppm trans-cinnmaldehyde+400 ppm carvacrol+100 ppm SDS) on *Salmonella* inactivation (a) and *Listeria* inactivation (b) during 3 independent trials of forced air cooling of cantaloupes. (Data are average and standard deviation of three repeats in each independent trial, treatment duration 180 min)

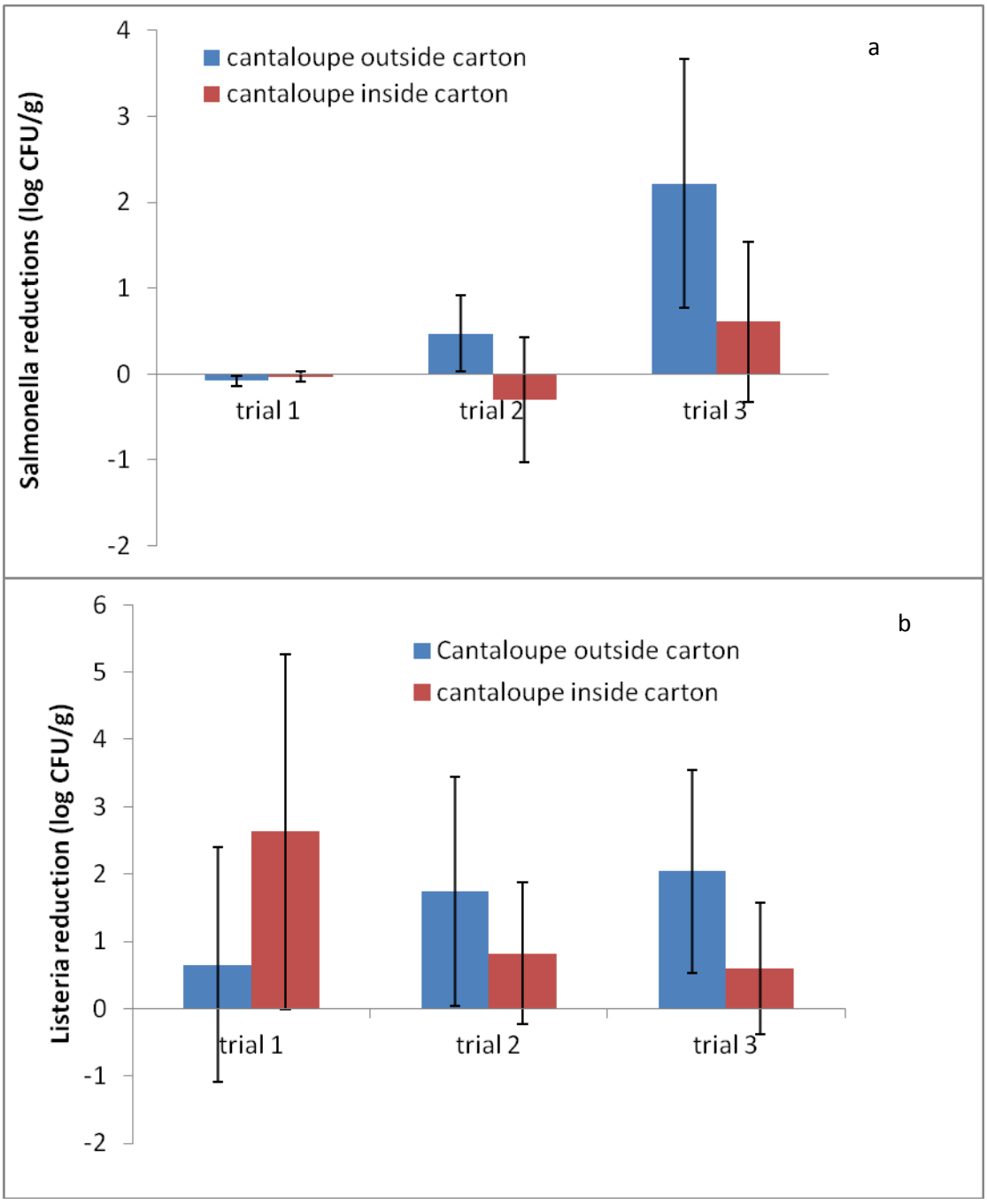
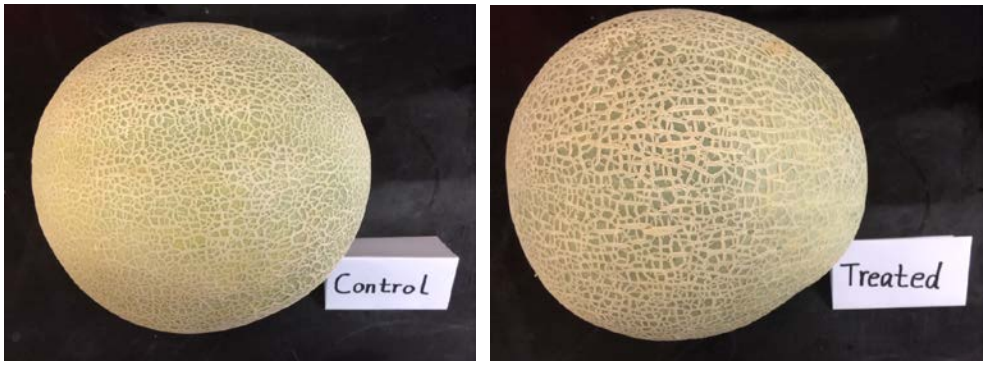


Table 1 Impacts of aerosolized chlorinated solution (Treated: Chs treatment, 200 ppm chlorine and pH adjusted using citric acid to 6.5 and 100 ppm SDS for 180 min) on the quality of whole cantaloupes stored at 4±1 ℃ for 10 days.

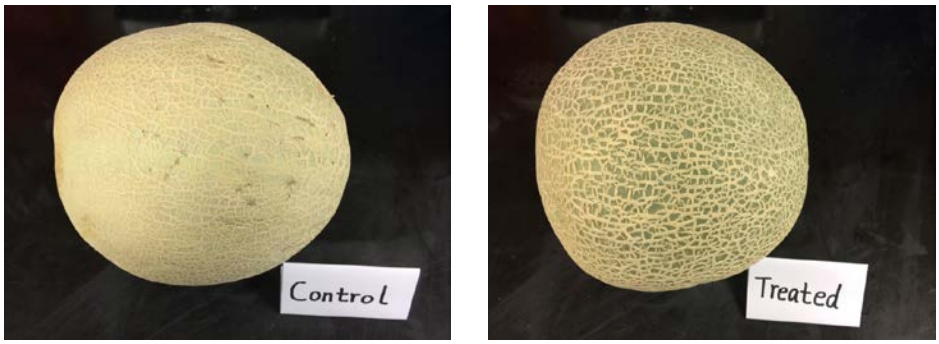
| | Day 0 | Day 1 | Day 5 | Day 10 |
|---|---|---|---|---|
| *Color (L*)* | | | | |
| Control | 59.95±0.21 | 64.65±3.46 | 63.2±1.98 | 64.4±2.12 |
| ChS treated | 65.35±3.32 | 62.45±0.07 | 61.45±0.21 | 62.4±3.54 |
| *Color (a*)* | | | | |
| Control | 17.5±0.14 | 14.9±4.53 | 18.85±0.35 | 18.2±2.69 |
| ChS treated | 17.6±2.12 | 18.1±0.71 | 17.8±0.14 | 18.35±3.46 |
| *Color (b*)* | | | | |
| Control | 32.9±0.28 | 35.6±0.57 | 37.25±1.34 | 36.25±4.17 |
| ChS treated | 35.4±1.27 | 35.7±0.28 | 33.95±0.64 | 36.5±5.66 |
| *Texture (N)* | | | | |
| Control | 9.98±1.29 | 7.77±2.15 | 3.57±0.95 | 5.31±2.04 |
| ChS treated | 14.21±5.81 | 9.46±1.06 | 5.09±2.35 | 5.72±0.89 |
| *Total soluble solids (°Brix)* | | | | |
| Control | 9.45±0.49 | 7.60±0.85 | 7.70±0.71 | 11.5±0.0 |
| ChS treated | 8.75±1.77 | 11.15±0.64 | 11.15±0.64 | 12.75±0.35 |
| *pH* | | | | |
| Control | 7.08 | 6.3 | 6.56 | 6.70 |
| ChS treated | 6.57 | 6.4 | 6.32 | 6.62 |
| *TPC (mg gallic acid/100 g FW)* | | | | |
| Control | 55.50 | 68.08 | 79.32 | 107.02 |
| ChS treated | 74.56 | 71.36 | 78.65 | 100.61 |
| *TBC (Log CFU/g)* | | | | |
| Control | 2.0±0.3 | 2.5±0.1 | 2.7±0.0 | 2.2±0.4 |
| ChS treated | 1.6±0.5 | 2.5±0.1 | 2.8±0.3 | 1.7±0.2 |
| *TYMC (Log CFU/g)* | | | | |
| Control | 0.0±0.0 | 0.0±0.0 | 0.3±0.0 | 0.2±0.0 |
| ChS treated | 0.0±0.0 | 0.0±0.0 | 0.2±0.2 | 0.0±0.2 |

Picture 1 Visual appearance for cantaloupes during the 10 day storage (Treated: Chs treatment, 200 ppm chlorine and pH adjusted using citric acid to 6.5 and 100 ppm SDS for 180 min; Control, no treatment)




Day 0


Day 1




Day 5


Day 10

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