CENTER for PRODUCE SAFETY

Center for Produce Safety 2013 RFP
Progress Report
September 30, 2014

Title of Research Project:
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

Principal Investigator:
Changqing Wu
Department of Animal & Food Sciences
University of Delaware
302-831-3029, changwu@UDel.Edu

Co-Principal Investigator(s):

Objectives

The overall objective of our proposed research is to develop a cost-effective sanitizing process which can be integrated into commercial forced-air cooling systems. The novel combination of ultrasonic atomization technology and antimicrobial formulations should reduce L. monocytogenes, Salmonella and/or spoilage organisms during the cooling of cantaloupes without affecting final quality and sensory attributes. The specific objectives of this proposal are:

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.

Accomplishments – *April 1, 2014 through September 30, 2014:* *Summarize activities performed and performance goals achieved for each research objective during this reporting period. Compare actual accomplishments with the goals established for the reporting period. Include significant contributions and role of project partners.*

There are five major accomplishments during the past 6 months. 1) Determined the impacts of treatment durations on *Salmonella* inactivation; 2) Determined the impacts of various antimicrobial formulations on *Salmonella* inactivation; 3) Determined the impacts of exposure locations on *Salmonella* inactivation; 4) Determined the impacts of surface structures on *Salmonella* inactivation**;** 5) Investigated the impacts of select treatments on *L. monocytogenes* inactivation.

As planned in objective 1, 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. 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 and 3. In addition, treatment durations ranged from 60 min to 360 min were evaluated (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). 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). Two different media were compared to determine the effects of media materials on pathogen 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).

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, 0.73 and 0.77 log CFU/g reduction respectively. While the 270 min Chs treatment had bigger *Salmonella (1.16 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 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.

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 possibilities (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 CFU/g log 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 summarized in Figs 2. Interestingly, higher inactivation efficacy was generally observed on grape tomatoes when compared with findings on cantaloupes, with exception of ES treatment. StorOx 2.0 at high and low concentrations decontaminated grape tomatoes by 1.5 and 1.0 CFU/g log reduction, respectively. PHAS inactivated approximately 0.8 CFU/g log of *Salmonella* on grape tomatoes. 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 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.

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, for both antimicrobial formulations, only data from one independent trial were used in the figure. The data from another independent trial suffered from low inoculations and big variation among the repeats, which made it difficult to calculate meaningful log reduction. A third independent trial is ongoing to confirm the findings.

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* population. We did not determine any *Salmonella* population in these uninoculated samples. Further evaluation will be also done on the uninoculated cantaloupe samples put adjacent to inoculated cantaloupe samples inside the same carton, as shown in plan for risk assessment below.

L. monocytogenes 390-1, an environmental isolate of *L. monocytogenes* associated with recent cantaloupe outbreak, was obtained from Dr. Kali Kniel, and used in some experiments. The data so far generally showed that the effective treatments could result in reduction of both pathogens. Furthermore, similar inactivation were observed between cantaloupe samples placed inside and those outside a “Regular” type of carton. More trials are ongoing to confirm these findings.

Plans - *October 1, 2014 through March 31, 2015 -* *Summarize activities and performance goals to be achieved during the next reporting period for each research objective. Include the role of project partners.*

In October, we will continue the optimization of treatments to inactive *L. monocytogenes.* Several trials will be also conducted to further enhance *Salmonella* inactivation by the effective treatments such as Chs. In addition, last planed activities in Objective 1 will be conducted. In these activities, four representative contamination scenarios will be designed to evaluate the risk factors and validate treatment efficacy during forced air cooling of cantaloupes. These different contamination scenarios will have different fruit contamination surface which might not have direct contact with moving air and the aerosolized antimicrobials.

All the protocols are now established to test the quality of cantaloupes, including sensory evaluation, color and texture analysis, measurement of titratable acidity, pH, total phenolics, soluble solids content, total aerobic bacterial plate counts, and yeast and mold counts. For the remaining time of this year, we will carry out experiments to accomplish the objective 2. We will thoroughly evaluate 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.

Problems or Delays - *Describe any unexpected delays, impediments, and challenges that have been encountered and actions you took to address these situations. If the work plan timeline, budget and/or methodology need to be adjusted, provide an outline of those requested changes. If there have been no problems or delays, note NONE.*

Due to unexpected cold winter weather and mechanic challenge for building the cooling system, our treatments were conducted in the unit two months later than initially planned. Large variations were also determined in some experiments using the cantaloupe samples, which made it difficult to quickly identify inactivation efficacy. More trials became necessary to confirm the findings. Experiments using grape tomatoes and XLD-N media were also conducted to determine whether produce surface

structure or enumeration media contributed to large variations, both of which were not initially planned and caused delay in our planned experiments.

In addition, as required by our University Environmental Health & Safety, we need to perform extensive decontamination between each trial because our experiments involve human pathogens. As a result, we make some changes on the work plan timeline and methodology, shown as table below. We have modified our approach to simultaneously evaluate the reduction of *L.monocytogenes* and *Salmonella* contamination during cooling of cantaloupes in our experimental system. We are also interested to test if flow rates have significant impacts on pathogen inactivation. We would like to request one month extension to finish our project.

To identify the best treatments on the reduction of *L.monocytogenes* or *Salmonella* contamination during cooling of cantaloupes in an experimental system.	PI, graduate students	Aug 2014--Oct 2014
To evaluate the risk factors and validate optimal treatment efficacy during forced air cooling of cantaloupes	PI, graduate students	Oct 2014--Dec 2014
To assess the effects of optimal treatment conditions on natural spoilage organisms, quality and sensory properties of cantaloupes after cooling of cantaloupes in a pilot-scale experimental system.	PI, graduate students	Nov 2014--Jan 2015
Data interpretation, extension and publication.	PI, graduate students	Oct 2014--Jan 2015

Funding Expended To Date

Summarize the level of grant funds expended to date. Do you expect to use all funds awarded to your project? If not, explain and estimate amount that will remain unexpended.

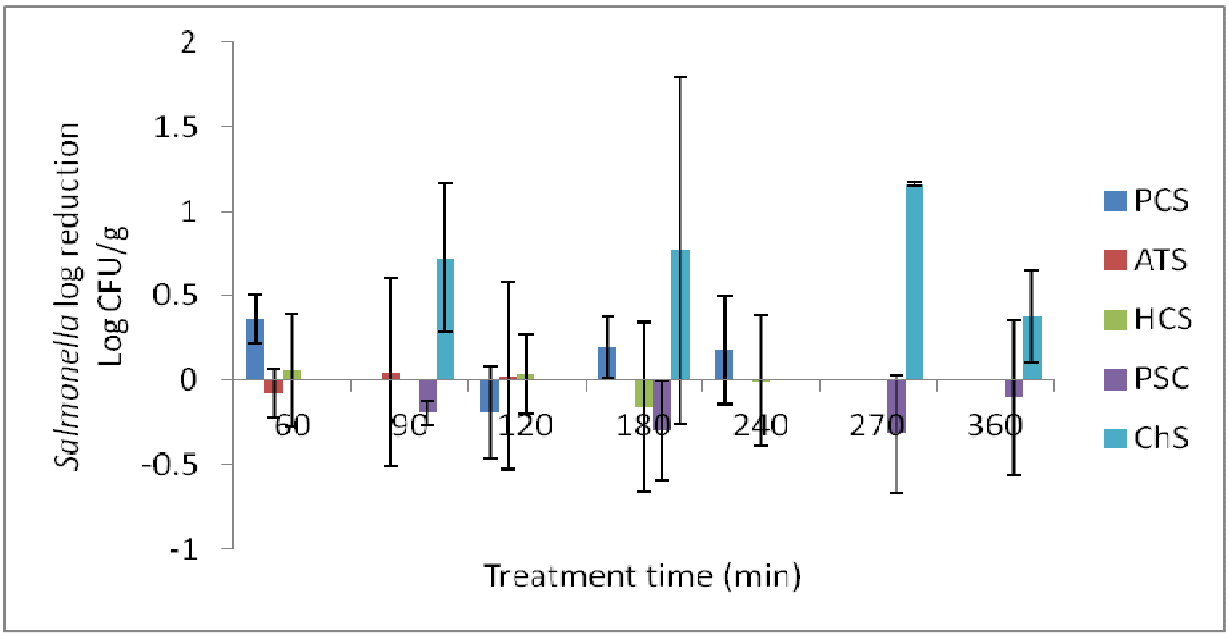
One fulltime graduate student is supported by the fund to conduct the proposed experiments. Partial tuition support at $8,000 has been provided by the funding for the graduate student. In addition, fund of approximately $ 3,030 was used to purchase lab supplies and cantaloupes. Recent purchase at $4,500 has not sent out to CPS yet. Yes, I expect to use all funds awarded to my project.

Do you have any requests of the Center for Produce Safety regarding this project?

Will we receive some California cantaloupes in a warehouse in this region in November? Because of natural microflora associated with cantaloupes harvested in Dr. Trevor Suslow's experiments, the cantaloupes developed molds quickly and were not usable for experiments, even all of them were shipped by Fedex overnight shipment. Since we are still working on the screening stages to identify the effective treatments, we just purchase fresh cantaloupes from market, without knowing the growth locations. But we are interested to determine the pathogen inactivation efficacy on California cantaloupes after we identified the most promising treatments in November.

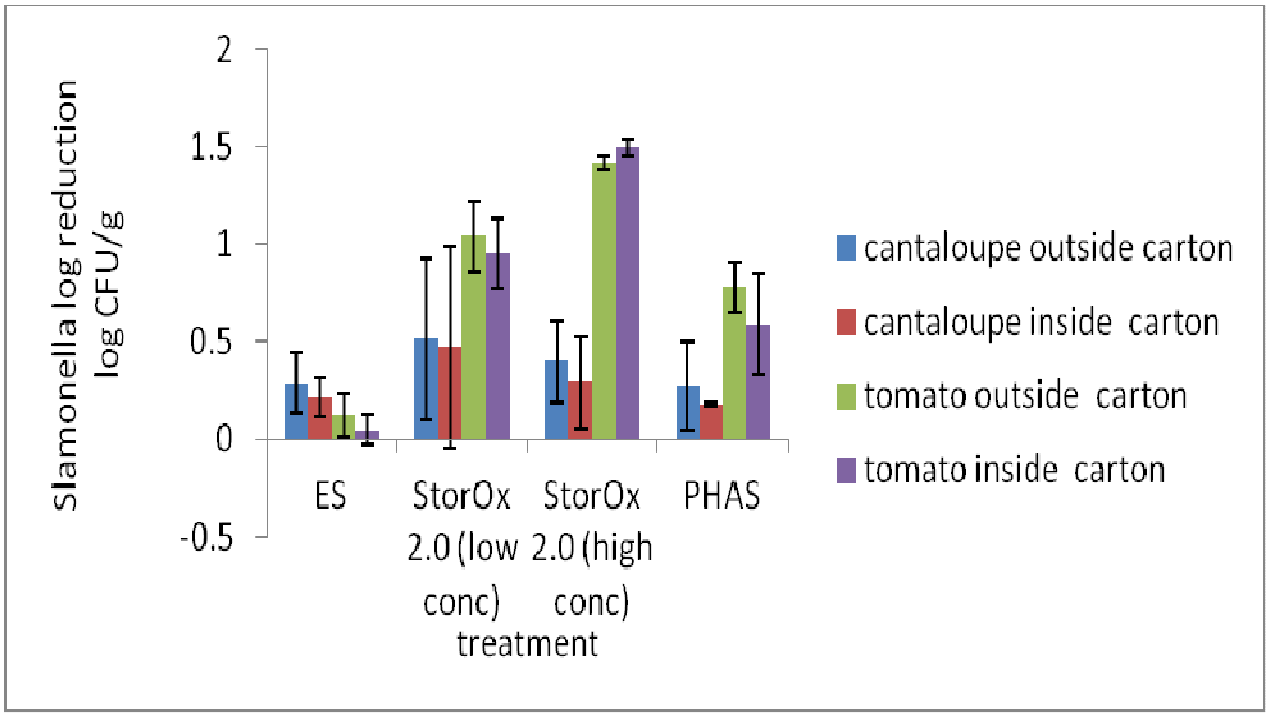
In commercial forced air cooling, one or several large permanently mounted fan delivers the forced air to be circulated through the vented cartons and around the packed fruit. Due to limitation of our system, the flow rate of our forced air fan could be far less comparable with the one used in commercial scale. I would like to have assistance from advisory board when we start the trials on flow rate. I will send out email to the board soon.

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)



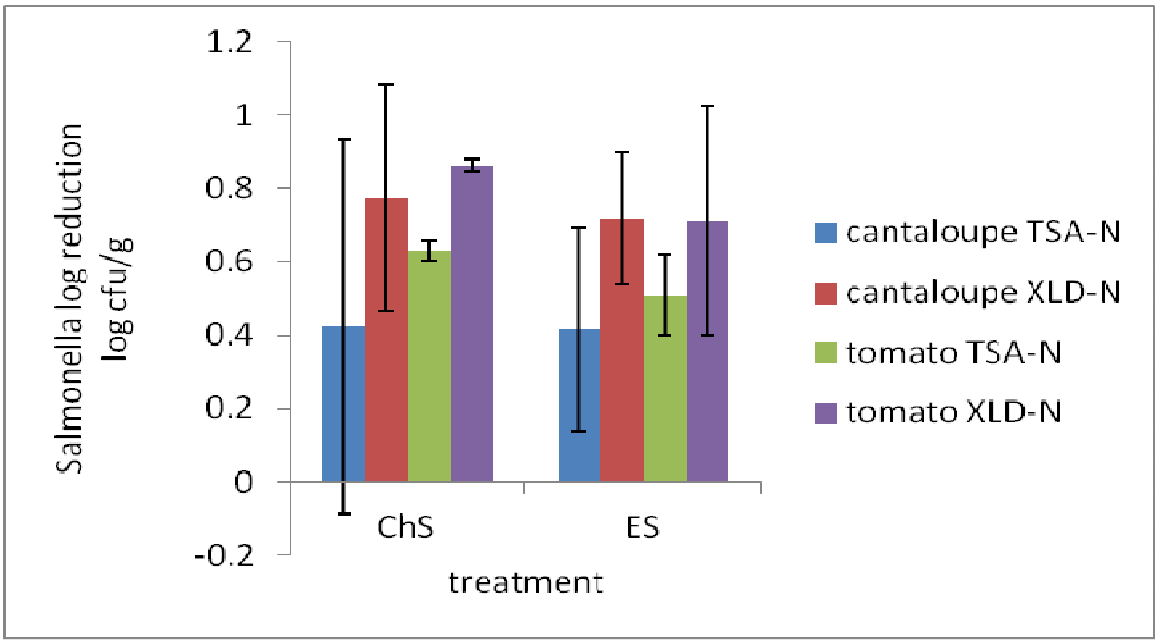
Note: PCS, 1 ppm peroxyacetic acid +1ppm *trans*-cinnamaldehyde+ 1ppm SDS;
ATS, 1 ppm acetic acid +1ppm thymol + 1ppm SDS;
HCS, 1 ppm hydrogen peroxide +1ppm *trans*-cinnamaldehyde+ 1ppm SDS;
PSC, 80 ppm peroxyacetic acid +100ppm SDS +100ppm *trans*-cinnamaldehyde;
Chs, chlorinated solution at 200ppm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

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 SDS.