CPS CENTER for PRODUCE SAFETY

CPS 2012 RFP
FINAL PROJECT REPORT

Project Title
Practical validation of surface pasteurization of netted melons

Project Period
January 1, 2013 – December 31, 2014 (NCE February 28, 2015)

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Objectives

1. *Evaluate the quantitative and qualitative survival of attenuated S. enterica and L. innocua during cantaloupe in controlled open-field experimental production.*

2. *Validation of postharvest mitigation treatments and effects of delays of cooling after sanitizing and temperature fluctuations during postharvest storage on pathogen survival.*

3. *Verify, by on-site studies, the efficacy of an evolving commercial process utilizing heat immersion as a rind pasteurization treatment across multiple time-points in each of two seasonal production intervals.*

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FINAL REPORT

Abstract

Cantaloupe rind washing achieved a partial surface pasteurization in a recirculating heated thermal-shower system was shown to achieve targeted bacterial reductions, relative to incoming populations on fruit, in model and commercial-scale systems. Over the two year project, several cycles of on-site testing and commercial system design and management were supported by the data developed at the cantaloupe packing operation and in laboratory validation studies. To better characterize the survival of Salmonella and Listeria on cantaloupe, simulating a contaminated irrigation event under field production conditions in California, surrogate strains were spot-inoculated onto the rind of fruit grown at the UC Davis research farm facility. Die-off from inoculated levels was rapid under high air temperature, high solar UV, and low humidity conditions typical of this production region. Surviving, recoverable populations were below the limit of detection within 14 days but at least 30% of fruit had detectable levels of survivors as determined by enrichment. Using desiccation-stress adapted cells in model systems and naturally-occurring environmentally stressed-adapted indicators in the commercial system, we have shown that tolerance to thermal-wash treatment at non-injurious time:temperature settings, alone, may be very high on the netted rind. However, microbiological reduction goals were achieved by a combined final process including a 60C thermal shower followed by a 30-50 ppm peroxyacetic acid spray and surface drying. To maintain these positive effects in microbiological reduction, fruit surface contact cleaning and sanitation downstream of the wash process is essential. Within these defined treatment parameters, no negative impacts on fruit quality were observed under optimal cantaloupe holding and distribution conditions. Extended storage periods or holding treated fruit at elevated and sub-optimal refrigerated storage conditions may result in accelerated water loss as compared to unwashed fruit. In an apparent cultivar associated manner, fruit washed by optimized thermal-shower treatments may result in reduced and delayed development of superficial molds compared to unwashed fruit or fruit exposed to injurious wash water temperatures. Overall, the outcomes of this study provide guidance for optimism if considering thermal-washing for netted melons as well as evidence for precaution and awareness in over-simplifying process design requirements and investment.

Background

The essential functionality of thermal surface-pasteurization of netted melons has been recognized at the basic and applied research level for over a decade, including vapor heat (steam), hot water immersion, and hot water brush-washing. Despite this evidence, commercial adoption by primary packers of the whole, raw commodity has been limited largely by cost and a limited compelling motivation. Various hot-water brushing and steam pasteurization technologies have been commercially installed at off-shore netted melon shipping points and in a few domestic fresh-cut processor faciities.

Following the mutlstate outbreak of Lis*teria monocytogenes* on whole cantaloupes in 2011 attributed to contamination at the packing operation, this prior research attracted attention in hopeful anticipation of solutions for the cantaloupe industry. A widely cited article in the blog-media, *Hot Water Bath Eliminates Pathogens on Cantaloupe*. James Andrews. Food Safety News (Feb 13, 2012), promoted potential adoption of the three-minute hot water immersion in 168F (75.5 ^{o}C). With the interest to convert this solely bench-top study to a high-throughput commercial system across extended seasons, growing districts, varieties, netted rind traits, fruit pulp temperature, and duration of pre-treatment storage, we were invited by committed adopters to participate in the development of validation data to support implementation decisions.

The purpose of this proposed research was to provide supportive lab-based data towards answering the following testable hypothesis;

Model Validation Criteria: *Hot water surface-pasteurization alone or in combination with a sequentially applied labeled disinfectant can achieve at least a 4-log reduction of applied pathogen surrogates in a controlled lab inoculation of netted melons and retain or improve fruit shelf-keeping properties.*

Commercial Validation and Verification Criteria: *Hot water surface-pasteurization alone or in combination with a sequentially applied labeled disinfectant can achieve at least a 2-log reduction of indigenous Total Coliform on netted melon rind and retain or improve fruit shelf-keeping properties.*

Prevention is the most important tool for addressing food safety goals during melon production. However, if cantaloupes are contaminated with human pathogens in the field, mitigation strategies, including postharvest killing steps, are needed to ensure the safety of this crop during its distribution or industrial processing. Off-loading of fruit to dump tanks for initial washing of cantaloupes in packinghouses, though no longer common in CA, is a common practice in many production areas. However, wash and product handling water, such as in dump tanks and flumes, may be a source of contamination or, more prevalently, a major point of cross-contamination. The re-circulated water systems of dump tanks and flumes often lead to accumulation of organic matter and can potentially become a vehicle of cross-contamination for incoming fresh product. The addition of antimicrobial agents to recycled water can inactivate bacterial cells and fungal conidia or spores, helping minimize cross-contamination. However, several studies have pointed out the ineffectiveness of chemical sanitizers to remove or inactivate foodborne pathogens, especially when they form biofilms on the rind surface. Microstructure of the netted rind gives the cantaloupe, and other netted melons, inherent surface roughness that favor bacterial attachment, acting as protection sites for bacteria as well as surface tension barriers to adequate sanitizer contact. *Salmonella* can attach itself to cantaloupe rind and may form resistant biofilms after 24 h; thus practical and effective strategies are needed to improve sanitizing treatments in order to penetrate protective rind sites and biofilms on the netted cantaloupe rind. As described above, hot water pasteurization has been proposed as a substantially improved alternative to melon disinfection with chemical sanitizers alone; however the majority of these studies have been developed under ideal laboratory conditions with contact times that, while potentially applicable to fresh-cut processing, have limited relation or reflection of industrial-scale conditions for commodity melons. Simply stated, while fresh cut processing can tolerate some degree of rind scalding and may be treating melons that are pre-chilled, this injury potential is too risky for retail melon marketing. In addition, the high costs associated with the maintenance of pasteurizing water temperature in heated immersion at commercial throughput levels clearly requires carefully assessment before widespread adoption can be recommended.

In addition to meeting food safety goals, killing heat shocks may also positively or negatively impact the post-treatment keeping quality of cantaloupes and other more sensitive netted melons. Therefore, during the early phases of adoption of this postharvest disinfection treatment, reaching a balance in which food safety objectives do not compromise the product quality must be considered and addressed to arrive at a set of Best Practice options. Sequential treatments, already in use by the melon industry, may allow the definition of a “safe window’ of heat that is equivalent to the current research reports but lower the requirement for

boiler capacity investment and recurring energy costs. The operational window may reduce injury by buffering the impacts of shelf-life reducing variables in fruit traits that always occur over seasonal and regional production that, realistically, have not been addressed.

This research was conducted to assess the efficacy of different postharvest mitigation treatments under controlled lab and evolving, industrial commercial systems, against artificially and environmental stress-adapted attenuated *S. enterica* and *Listeria innocua* as surrogate of *L. monocytogenes*, during postharvest handling of whole cantaloupe. With the participation of industry cooperators, we assessed the efficacy of the work-in-progress design and implementation of a high-throughput commercial system. In doing this, developed knowledge and practical experience can be transferred, as one option for rind disinfection, and implemented by interested cantaloupe industry shippers. We anticipate these results would also be extendable to other durable fruit categories.

Research Methods and Results

METHODS

Produce. Cantaloupes were grown for field or laboratory inoculation at the Plant Sciences Research Farm Facility located on the land-grant property of the University of California, Davis. At full-slip maturity, cantaloupes for model studies were harvested and used within 2h for laboratory experiments involving thermal and antimicrobial rind pasteurization of native microbiota and field-inoculated bacteria. Additionally, field-packed melons that were used for laboratory inoculations, subsequently subjected to different wash processes to evaluate surface-disinfection, were obtained directly from a local wholesaler and immediately transferred to a 2.5˚C walk-in cooler for use within 2 days.

Inoculation. Stock cultures of an attenuated *Salmonella* Typhimurium (*att*PTVS 337; a rifiampicin-resistant derivative of *S. enterica* sv. Typhimurium χ3895) and *Listeria innocua* (TVS 451; a rifampicin-resistant derivative of ATCC® 33090™) were used in field and model wash line studies. In earlier and related project work, we demonstrated that these permitted nonpathogenic strains had sufficient fitness to be reasonable surrogates for pathogenic isolates. Isolates were maintained at -80˚C and aseptically streaked onto trypticase soy agar plates (Difco, Becton, Dickinson, & Co., Sparks, MD) amended with rifampicin (Fisher Scientific, Waltham, MA) (80 mg L^{-1}) for incubation at 37˚C for 24 h and 48 h, respectively. Lawns of each strain were prepared on TSA-R for 24 h of incubation at 37˚C. Cultures were independently resuspended in sterile Butterfield's Phosphate Buffer (Whatman, Florham Park, NJ) (BPB).

For laboratory inoculations, melons were held at 22˚C for 18 h prior to inoculation. 100 µl of 7 log CFU/ml suspensions of each strain were spot-inoculated in two 5-cm diameter circles per melon (total of four circles per melon) and allowed to dry for 4 h in a vented container at 22˚C. Melons were held 3 d at 2.5˚C prior to washing to allow populations to reach a steady state of low metabolic activity and desiccation-stress adaptation.

For thermal $\pm$ antimicrobial rind pasteurization studies using field-inoculated cantaloupe, replicates of individual fruit, near harvest maturity, were similarly spot-inoculated on the upper rind-surface at dusk to allow inoculum to dry will limited negative impact of solar-UV inactivation. Using this procedure, a slower rate of desiccation also maintains viable populations of applied bacteria at fairly reproducible target end-populations. To assess survival in the field (Objective 1) two replicated experiments of 45 melons per pathogen surrogate were spot-inoculated with

100 µl of 8 log CFU/ml *Salmonella* Typhimurium (PTVS 337) and *Listeria innocua* (TVS 451) in two separate 10-cm diameter circles (A = 19.63 cm^2).

At 24 h, 4, 7 and 14 d post-inoculation, 10 melons were removed from the field and the inoculated circles from each melon were removed with a sterile knife, added to 10 ml KPO_4+Tween, and massaged vigorously to remove attached bacteria. Samples were quantified for *Salmonella* and *L. innocua* by surface-plating on CHROM *Salmonella*+rif and CHROM *Listeria*+rif, respectively, following incubation at 37˚C for 24 and 48 h.

If samples are not quantifiable (no characteristic colony growth), 10 ml 2X BPW+rif or 2X LEB+rif were added to the appropriate sample bags with wash buffer and the original rind disc, and incubated at 37˚C for 24 and 48 h, respectively. Aliquotes of these enrichment cultures were spot-plated on CHROM Sal+rif and CHROM Lis+rif.

Inoculated cantaloupe remained attached to the vine in the field for three days prior to harvesting and immediate use in wash process evaluations, simulating commercial operations.

Laboratory-scale thermal-shower wash. A recirculating heated wash system consisting of a 0.61 x 0.61 x 0.46 m sink, thermally protected centrifugal pump (Model 1103007488, Royersford, PA), Hot Tap Pro 100 water heater, and perforated shower-manifold, consisting of a parallel array of three polyvinyl chloride pipes was used for laboratory-scale experiments. Five noninoculated or inoculated melons each were either: 1) washed for 45 sec with 63-68˚C water, 2) pressure sprayed for 1 sec, adequate fir full coverage of the inoculated spots, with 30 ppm peroxyacetic acid (SaniDate 5.0; BioSafe Systems, LLC., East Hartford, CT), 3) washed for 45 sec with 63-68˚C water followed by 1 sec pressure spray with 30 ppm PAA, or 4) retained without water contact to determine initial populations of bacteria. All fruit were air-dried (determined visually, replicating commercial systems) by placement in front of a large fan prior to microbiological analysis.

Two 5-cm diameter circles from each noninoculated melon or the two circles inoculated with *S.* Typhimurium or *L. innocua* were removed using a sterile knife and hand-massaged in 20 ml of potassium phosphate buffer (EMD Chemicals, Inc., Gibbstown, NJ) amended with Tween 20 (Fisher Scientific, Fair Lawns, NJ). Samples were appropriately diluted in sterile BPB and surface-plated on CHROMagar *Listeria* or CHROMagar *Salmonella* Plus (CHROMagar, Paris, France), both amended with rifampicin (Fisher Scientific, Waltham, MA) (80 mg L^{-1}) and pyruvic acid sodium salt (Fisher Scientific) (1 g L^{-1}) (Sal-RPyr and Lis-RPyr, respectively), for recovery of *L. innocua* and *S.* Typhimurium after 48 and 24 h of incubation at 37˚C, respectively. Total heterotrophic bacteria were enumerated from noninoculated rinds using Plate Count Agar (Difco, Becton, Dickinson, & Co., Sparks, MD) amended with pentachloronitrobenzene (Amvac Chemical Corp., Newport Beach, CA) (5 mg ml^{-1}) and pyruvic acid sodium salt (48 h incubation at 29˚C) with total coliforms enumerated using CHROMagar ECC amended with pyruvic acid sodium salt (24 h incubation at 37˚C). *Enterobacteriaceae* were enumerated using Petrifilm™ *Enterobacteriaceae* Count Plates (3M™, St. Paul, MN) (24 h incubation at 37˚C). Inoculated samples below the limit of spread-plate detection (-0.34 log CFU/cm^2) were enriched with an equal volume to sample enrichment aliquot (20 ml) of double strength Buffered Peptone Water (Difco, Becton, Dickinson, & Co.) or *Listeria* Enrichment Broth (EMD Millipore Corp., Billerica, MA) amended with rifampicin (160 mg L^{-1}) for *Salmonella* and *L. innocua*, respectively. Following incubation at 37˚C, three 33-µl samples were spot-plated on Sal-RPyr and Lis-RPyr and incubated further to determine presence/absence of the inoculated bacteria on the rind surface.

Assessment of a Revised Rind Sampling Protocol. Due to several logistic and staffing factors for sample collection and sample processing associated with on-site commercial facilities, three assessments of modified protocols for simplified enumeration of naturally-occurring bacteria on cantaloupe rinds at harvest were conducted. Briefly, in prior project periods, we collected hundreds of whole cantaloupe from incoming, in process at different stages, and final packed fruit which typically resulted in significant handling issues in the lab but limited variability in bacterial populations. Handling the number of whole melons desired for this project also created significant waste removal costs which became a limiting consideration in 2014. To streamline management of this objective, either two, six, or twelve rind discs (5 cm^2) were excised with a sterile metal coring-device from three to five cantaloupes harvested directly from commercial fields on each date corresponding to different CA fields, varying seasonal conditions, and crop management practices. Rind discs were placed in buffer solutions and hand-massaged, as described above. Total heterotrophic bacteria were enumerated from these noninoculated rinds using Plate Count Agar amended with pentachloronitrobenzene and pyruvic acid sodium salt (48 h incubation at 29˚C). Total coliforms were enumerated using CHROMagar ECC amended with pyruvic acid sodium salt (24 h incubation at 37˚C). *Enterobacteriaceae* were enumerated using Petrifilm™ *Enterobacteriaceae* Count Plates (3M™, St. Paul, MN) (24 h incubation at 37˚C).

Commercial facility hot wash. At a high-throughput commercial packing facility in CA, multiple cantaloupe and water samples were collected on two occasions during normal daily mid and late-season operations in 2014. At each of four 30-min intervals, 10 melons were collected at various unit-operational points of the receiving and packing line: prior to wash, after 65-70˚C thermal-shower wash, after 65-70˚C thermal-shower wash and a subsequent 45-50 ppm peroxyacetic acid (PAA) spray applied immediately after exiting the thermal-shower tunnel, and following passage through the air-drying tunnel and conveyor transfer to the final packing stations. Two 5-cm diameter circles were sampled from each melon for total heterotrophic bacteria, total coliforms, and *Enterobacteriaceae* as described in section 1.3.

Water samples were collected at each of the four 30-min intervals from both the thermal-shower tunnel at the perforated stainless steel pan and at the return water recirculation tank prior to the heat-exchange boilers. Water samples were assessed for temperature, pH, oxidation-reduction potential, chemical oxygen demand, turbidity, and electroconductivity. Samples for microbial analysis were immediately stored on ice for transportation back to the lab, and then assessed for populations of total coliforms and *E. coli* using the Quanti-Tray 2000 system (IDEXX Laboratories, Westbrook, ME) (24 h of incubation at 37˚C). Water samples were filtered through 0.45 µm membranes using ISO-GRID/NEO-GRID Membrane Filter Systems (Neogen Corp., Lansing, MI) and placed on Violet Red Bile Glucose Agar (Neogen Corp., Lansing, MI) (24 h incubation at 37˚C) for enumeration of *Enterobacteriaceae*.

Melon quality in storage post-treatment. To assess positive of negative impacts on fruit quality, twenty-nine melons of three cultivars - Fiji, Dynamic, and Caribbean Gold - were submerged for 45 sec in a heated water bath set at either 70 or 80˚C. Based on the cooperator system design and practical requirements for fruit throughput, 45 sec was the maximum time of exposure at which the system could operate. These temperatures were selected, earlier in the project period, as the upper limit of non-injurious time:temperature treatment which provided at least a 3-log reduction in applied pathogenic strains to cantaloupe rind and at least a four-log reduction of these bacteria suspended in the wash water. Melons were weighed immediately following heated water immersion and air-drying and after 14 d of storage at 7.5˚C, with each fruit assessed for presence of mold and soft/sunken discolored spot development at the end of storage. A holding temperature of 7.5C was selected to simulate an accelerated shelf-keeping

quality assessment requested by our commercial partners based on early observations and experience with the thermal wash process outcomes during commmecial distribution. In a second storage study, ten Fiji and ten Dynamic melons were submerged for 45 sec in 70, 80, or 95˚C water and stored for 28 d at 7.5˚C, after which melons were re-weighed and assessed for the presence of mold. In the final storage study, 40 cantaloupes each (cultivar Dynamic) were either: 1) submerged for 45 sec in 65˚C water, 2) submerged in 65˚C water followed by 1 sec of 50 ppm PAA spray, or 3) submerged for 45 sec in water containing 35 ppm free chlorine (pH 6.5). Melons were weighed immediately after treatment and again after storage for 14 d at 2.5 or 7.5˚C, with fruit additionally assessed for presence of mold and soft/sunken discolored spot development.

On-site Process Control Verification. In addition to setting process control verification options based on microbiological criteria, we used infra-red imaging to assess the uniformity of surface and sub-surface heat penetration of cantaloupe in model and commercial studies. At hand-held digital infrared imaging camera (FLIR Systems Inc.) was used, following manufacturer's instructions and software, to demonstrate this approach to conduct either non-destructive or destructive sampling assessments during daily operations at a commercial packing facility.

RESULTS

Survival of attenuated *S. enterica* and *L. innocua* on cantaloupe in an open-field environment. In each field study, the applied bacteria declined rapidly **with**in the first few days under prevailing summer climatic conditions in Davis, CA which are very representative of main season cantaloupe production in CA. The surrogate *Salmonella* had a greater quantitative persistence than the *L. innocua* up to seven days post-inoculation but both remained detectable on 30-50% of melons at 14 days (Figure A).

Assessment of a Revised Rind Sampling Protocol. The degree of variability among an analysis of 2, 6, or 12 rind discs was sufficiently similar to the bacterial population variability observed during enumeration of whole cantaloupe washes for total heterotrophic bacteria, total Enterobacteriaceae, and total coliforms. Given this outcome, during 2014-2015 we elected to use 2 discs from replicated cantaloupes for assessing the efficacy of different thermal/antimicrobial wash process treatments at the commercial packing facilities. An example of the outcome for a five-replicate study is provided in Table A.

Laboratory-scale hot wash. Application of the 30 ppm PAA spray alone resulted in no significant reduction ($P > 0.05$) in any populations native to cantaloupe rinds compared to the unwashed controls, whereas populations of lab-inoculated *S.* Typhimurium and *L. innocua* were significantly reduced ($P < 0.05$) by 2.06 and 1.88 log CFU/cm^2, respectively (Table B). For all native and inoculated species, sequential application of PAA following the thermal wash did not result in increased bacterial inactivation ($P > 0.05$) compared to the thermal wash alone.

When treated with a 30 ppm PAA spray alone, 100% of melons were positive for both *S.* Typhimurium and *L. innocua*, whereas 70 and 10% were positive for the strains, respectively, following the 65˚C hot wash. Of the melons exposed to both treatments sequentially, 40 and 100% were positive for *L. innocua* and *S.* Typhimurium, respectively.

Commercial facility thermal-shower wash. For the most part, no significant differences (P > 0.05) in bacterial populations on melons were observed between the sampling time points; populations on melons collected from each location were averaged together for analysis and can be seen in Figure B. For all organisms, populations on melon rinds decreased significantly (P <0.05) following the 65˚C hot wash and 50 ppm PAA spray compared to incoming melons, with average reductions of 1.54, 2.09, and 1.93 log CFU/cm^2 for total heterotrophic bacteria, total coliforms, and *Enterobacteriaceae*, respectively (Figure A). The application of the 50 ppm PAA spray following the hot wash did not result in additional reductions (P > 0.05) for any of the bacterial populations studied compared to the heated wash alone. However, significant increases (P < 0.05) in all populations (1.27, 2.04, and 1.57 for total heterotrophic bacteria, total coliforms, and *Enterobacteriaceae*, respectively) were observed on melon rinds between the PAA spray and final pack, indicating the potential for re-contamination of melons via equipment surfaces in the packing line.

Microbial populations in both the thermal-shower tunnel and recirculation tank were statistically similar (P > 0.05) throughout daily wash-pack operations, ranging from 1.44-2.53 log MPN/100 ml for total coliforms, *E. coli*, and *Enterobacteriaceae* (Table C). Wash water parameters were very similar between samples taken from the shower and recirculation tank (Table C). In prior year water samples our results indicated the need for improved filtration of particulates, primarily cantaloupe netted rind material, due to the overall increase in viable bacteria during daily operations. A series of 'sock-filters' was added for the 2014 season and appears to have been responsible in the consistency of low levels of bacterial recovery from process water across incoming loads.

On-site Process Control Verification. Examples of infrared thermal imaging is provided in the Appendix as Figures C & D.

Melon quality in storage. No significant changes in percent weight loss were observed for Caribbean Gold melons after 14 d of storage at 7.5˚C, whereas Fiji and Dynamic melons both displayed significantly greater weight loss when washed with 80˚C water compared to unwashed controls (Table D). When softening or mold development occurred, Dynamic and Caribbean Gold melons were observed to develop primarily black mold and soft spots at localized areas on the fruit, whereas Fiji melons tended to develop grey-green mold and overall softening of the entire fruit. Treatment with 70 or 80˚C water resulted in a lower percentage of Dynamic melons exhibiting mold development (0 and 40%, respectively) compared to the unwashed controls (100%) (Table E) after 28 d of storage at 7.5˚C. However, the 95˚C wash was associated with a greater number of melons exhibiting mold (90%), likely due to its damaging effect on the melon rind. Although no apparent difference in mold development was observed between recommended (2.5°C) and suboptimal (7.5°C) storage temperatures, storage at 2.5˚C resulted in lower average soft spot development for all wash treatments (Table F). At both storage temperatures, melons treated with chlorinated water displayed slightly greater soft spot development than melons exposed to the thermal-wash or thermal-wash with PAA spray.

Outcomes and Accomplishments

Overall, we have provided quantitative and qualitative data under laboratory, model system, and commercial system operations which, collectively, highlight key and critical practical elements of thermal wash process design and management for cantaloupe. Laboratory studies conducted early in the project period largely confirmed anticipated outcomes for effective surface disinfection of the netted rind using a heated wash process and more substantial inactivation of free-floating bacteria, both naturally-occurring and inoculated, to minimize cross-contamination potential during commercial handling. However, it was also demonstrated that these systems

have inherent complexities and the potential for negative quality impacts, if the process controls are not stringent, that present some real challenges under commercial system management. A key consideration determined over the two year program was the importance of removing suspended solids from the recirculating heated water by filtration to prevent bacterial load accumulation if no effective water chemical sanitizer is used at temperatures less than 80C due to the practical restrictions on residence time for each cycle.

Summary of Findings and Recommendations

With proper system design and management, we find that a thermal-shower partial pasteurization of the netted rind of cantaloupe, though costly to implement on a large scale in a manner to most closely reflect the supporting validation studies, is possible to achieve without compromising quality. It was not possible, nor anticipated to be a project outcome, that any verification standard could be designed for validated or verifiable reduction of naturally-contaminating Salmonella or Listeria. In a separate survey study within the same project period, our lab tested over 4,000 cantaloupes collected from CA shippers after packing, including more than 400 from our cooperator's facility, and found no detectable presence of either pathogen. Therefore, our studies focused on the more ubiquitous general bacterial indicators but still found limited levels and consistency in log reduction, relative to lab validation outcomes. Based on our outcomes in assessing the thermal wash temperature tolerance of native indicators compared to applied pathogens, we recommend using total Enterobacteriaceae as the routine process verification standard in addition to water temperature monitoring. Infrared imaging is a viable nondestructive option for monitoring the uniformity and peak rind temperature exposure of the process. Regardless, commercial handlers should carefully evaluate this option before embarking on installation of system based on thermal washing alone or in combination with an approved adjunct antimicrobial process aide.

APPENDICES

Publications and Presentations (required)

No publications to peer-review journals have been submitted at this time but several are planned for the near future. The laboratory validation and verification study data from early project outcomes, already provided in Progress Reports, will be combined with the current outcomes in the final process system for future publications.

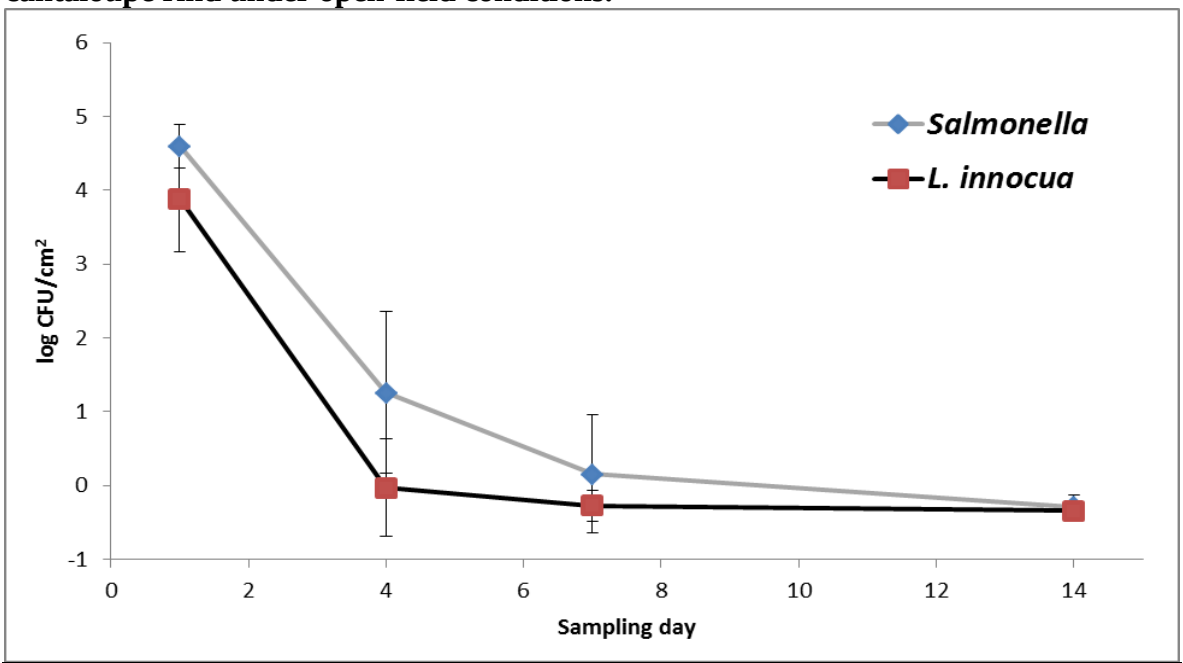
More than 40 presentations specific to or including this research project during its progress have been made at diverse produce industry and food protection forums nationally and internationally. Specific annual updates were made to the CA Melon Research Board and at the CPS Symposium. Presentations of this project in progress were made annual at technical sessions and a cantaloupe food safety Symposium at the International Association of Food Protection.

Budget Summary (required)

The allocated funds for this project have been expended in the execution of the planned and modified objectives associated with this project.

Tables and Figures (optional)

Figure A. Persistence* of surrogate attenuated *Salmonella* and *Listeria innocua* on cantaloupe rind under open-field conditions.



Sampling day	*Salmonella*			*L. innocua*		
1	4.60 ± 0.30	A	*100*	3.88 ± 0.71	A	*100*
4	1.26 ± 1.10	B	*100*	-0.03 ± 0.66	B	*80*
7	0.16 ± 0.80	C	*90*	-0.27 ± 0.20	B	*80*

14	-0.29 ± 0.17	C	*50*	-0.34 ± 0.00	B	*30*

***** In the figure-associated data table, the italicized numbers correspond to the percent positive enrichments of the ten total individuals assayed qualitatively for presence/absence of surviving applied bacteria at each time-point.

Figure B. Populations of total heterotrophic bacteria, total coliforms, and *Enterobacteriaceae* on cantaloupe rind throughout daily packing operations at a commercial facility.

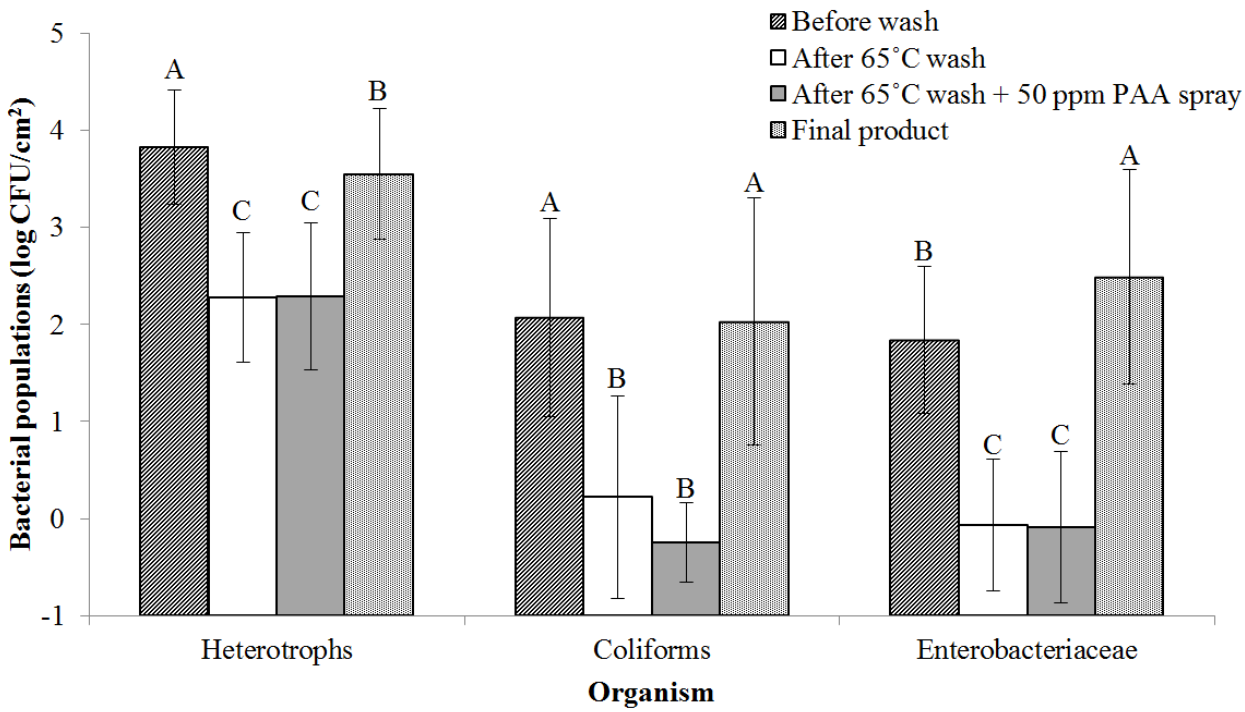


Table A. Example from one of three assessments of rind core quantification of indicator bacteria on cantaloupe rind for simplified enumeration of naturally-occurring bacteria on cantaloupe rinds at harvest and following wash processing

		Bacterial populations (log CFU/cm^2) **Limit of Detection** -0.34 log CFU/cm^2		
Melon	**Sample**	**Total plate count**	**Total coliforms**	***Enterobacteriaceae***
A	2-circle samples	2.71	0.75	0.49
		3.13	-0.34	0.01
		2.72	-0.29	0.61
	6-circle	2.61	-0.34	-0.29
B	2-circle samples	2.91	-0.34	0.01
		3.32	0.41	0.96
		3.77	1.40	2.15
	6-circle	5.01	1.24	2.06
C	2-circle samples	3.09	2.21	2.32
		3.16	1.44	1.93
		3.38	0.31	0.88
	6-circle	3.36	-0.29	0.49
D	2-circle samples	2.63	-0.34	-0.29
		2.90	0.94	1.33
		2.90	0.96	1.35
	6-circle	2.66	-0.34	0.41
E	2-circle samples	3.02	0.75	1.44
		4.08	2.69	2.77
		3.99	2.87	2.85
	6-circle	3.53	2.30	2.31

Table B. Bacterial populations after exposure to various treatments on non-inoculated melons or melons inoculated in the lab to contain ~3.5-4 log CFU/cm^2 *S.* Typhimurium or *L. innocua*.

Organism	Bacterial populations on melon rinds (log CFU/cm^2) *(% Positive Enrichments)*							
	Initial		65°C wash		30 ppm PAA spray		65°C wash + 30 ppm PAA spray	
Total heterotrophic bacteria	3.52 ± 0.32 AB [a]	--	2.42 ± 0.54 C	--	3.57 ± 0.17 A	--	2.81 ± 0.23 BC	--
Total coliforms	1.62 ± 0.83 A	--	0.70 ± 1.27 A	--	2.27 ± 0.73 A	--	1.09 ± 1.16 A	--
Enterobacteriaceae	2.05 ± 0.69 AB	--	0.35 ± 0.64 C	--	2.46 ± 1.05 A	--	0.76 ± 0.76 BC	--
S. Typhimurium	3.49 ± 0.50 A	*(100)*	0.22 ± 0.49 B	*(100)*	1.43 ± 1.03 B	*(100)*	0.65 ± 0.62 B	*(77)*
L. innocua	3.86 ± 0.29 A	*(100)*	0.49 ± 0.72 C	*(92)*	1.98 ± 0.44 B	*(100)*	1.14 ± 0.37 BC	*(92)*

[a] Means with different letters represent bacterial populations that differ in significance ($P < 0.05$) within a row.

Table C. Physicochemical parameters and bacterial populations in wash water samples collected from the shower and recirculation tank at a commercial packinghouse.

Water sample	Wash water parameters						Bacterial counts (log MPN/100 ml)		
	Temp (˚C)	pH	ORP (mV)	COD (mg O_2/L)	Turbidity (NTU)	EC (mS)	Total coliforms	*E. coli*	*Enterobacteriaceae*
Shower	62.7 ± 1.4	8.59 ± 0.68	344 ± 190	1556 ± 131	249 ± 28	1.06 ± 0.03	2.45 ± 0.20	1.52 ± 0.28	2.41 ± 0.20
Recirculation Tank	65.7 ± 2.6 [a]	8.37 ± 0.78	254 ± 189	1439 ± 75	237 ± 28	1.11 ± 0.07	2.45 ± 0.26	1.44 ± 0.34	2.53 ± 0.19

[a] Wash water parameters of recirculation tank water were only assessed during one sampling day.

Table D. Percent weight loss of melons after 14 d of storage at 7.5˚C.

Hot water treatment	Melon weight loss (%)		
	Cultivar		
	Fiji	Caribbean Gold	Dynamic
No wash	3.86 ± 0.95 B [a]	4.21 ± 0.86 A	3.33 ± 0.57 B
70˚C wash	4.84 ± 0.86 AB	5.22 ± 1.21 A	4.72 ± 0.47 A
80˚C wash	5.66 ± 1.39 A	5.07 ± 0.93 A	4.96 ± 0.55 A

[a] Means with different letters represent weight loss that differed in significance ($P < 0.05$) in terms of hot water treatment for each cultivar.

Table E. Effect of 28 d of storage at 7.5˚C on percent weight loss and mold development of Fiji and Dynamic melons.

Hot water treatment	Storage effects on melons					
	Cultivar					
	Fiji			Dynamic		
	Weight loss (%)		Melons exhibiting mold development (%)	Weight loss (%)		Melons exhibiting mold development (%)
No wash	5.81 ± 1.04	B [a]	70	6.90 ± 2.52	B	100
70˚C wash	7.69 ± 1.48	B	0	7.59 ± 1.48	AB	0
80˚C wash	7.35 ± 1.08	B	20	7.73 ± 3.38	AB	40
95˚C wash	10.20 ± 3.45	A	40	10.38 ± 2.77	A	90

[a] Means with different letters represent weight loss that differed in significance ($P < 0.05$) in terms of treatment for each cultivar.

Table F. Effects of 14 d of storage at 2.5 or 7.5˚C on qualities of Dynamic melons exposed to hot wash, PAA, and chlorine treatments.

Storage temperature	Treatment	Storage effects on Dynamic melons			
		Weight loss (%)		Fraction of melons exhibiting mold	Average soft spots per melon
2.5˚C	65˚C wash	3.13 ± 0.95	A [a]	3/17	0.18
	65˚C wash + 50 ppm PAA spray	3.57 ± 0.70	A	0/18	0.56
	35 ppm Cl	2.98 ± 0.92	A	0/20	1.45
7.5˚C	65˚C wash	5.14 ± 1.53	B	1/17	2.29
	65˚C wash + 50 ppm PAA spray	6.58 ± 1.67	A	1/18	2.24
	35 ppm Cl	5.36 ± 1.75	AB	2/20	5.15

[a] Means with different letters represent weight loss that differed in significance ($P < 0.05$) in terms of treatment for each storage temperature.

Figure C. Surface Rind Temperatures of whole (upper) or cross-sectioned cantaloupes following 45 sec residence in the thermal-shower at different water temperatures.

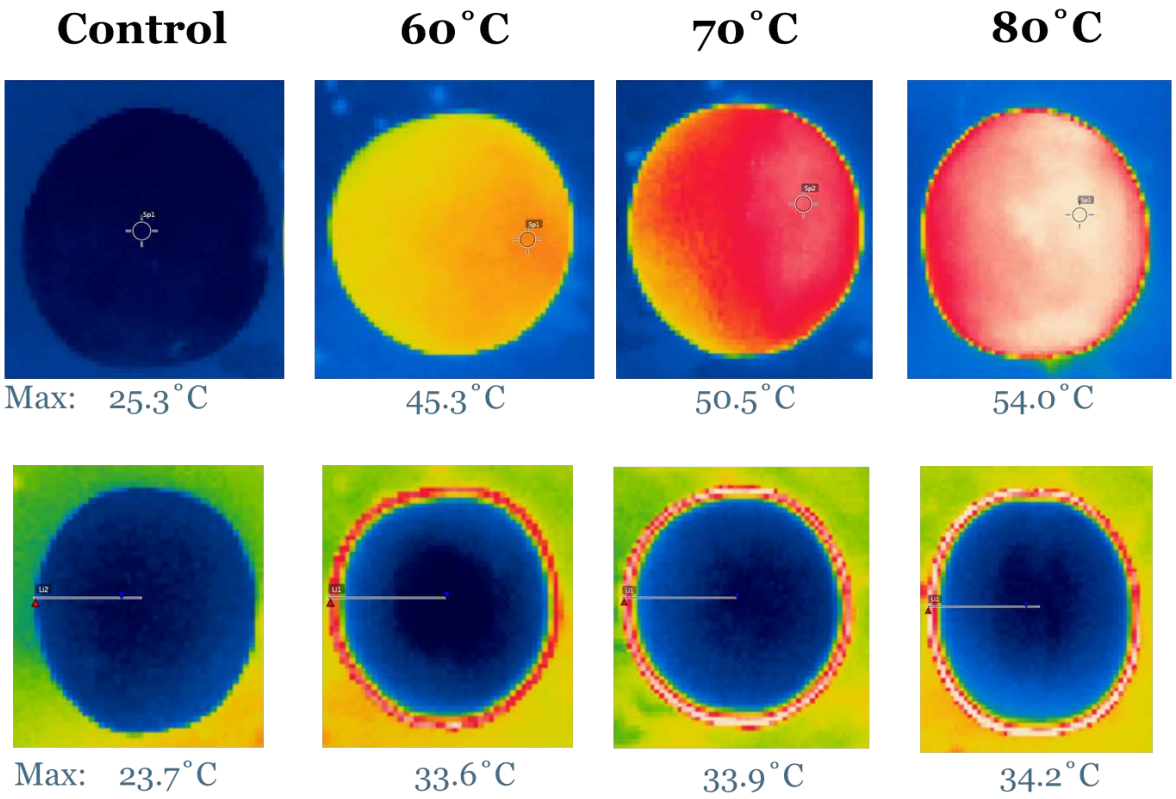
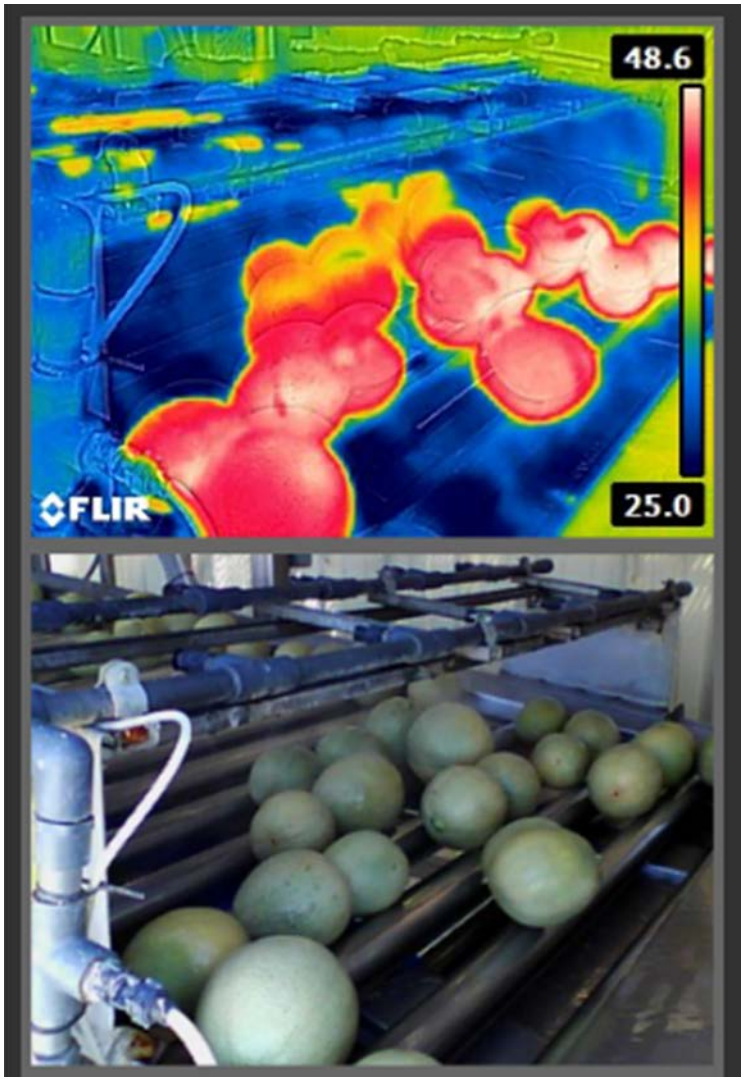


Figure D. Surface Rind Temperatures of whole cantaloupes emerging from the thermal-shower chamber following 15 sec residence at a 60C water temperature.



Suggestions to CPS (optional)

This was a very complex but instructive research project. A post-mortem analysis of factors which facilitated or limited achieving planned research goals is underway and CPS may choose to participate with a view to further refining project proposal assessments during funding consideration and in-progress support or facilitation.