RESEARCH IN PROCESS

Control of *Listeria monocytogenes* on Apple Through Spray Manifold-Applied Antimicrobial Intervention

SUMMARY

The Pacific region apple industry suffered a significant loss of income following the recent *Listeria monocytogenes* outbreak associated with caramel apples. The final FDA Produce Rule and Preventive Controls Rule are challenging apple packers and handlers to develop specific efficacy data for their process controls. The apple industry has an immediate need to begin the process of science-based improvements in *Listeria* control during packing and subsequent storage. The overall goal of the proposed studies is to comparatively assess and validate critical operating parameters for registered, commercially practical, and legally allowed sanitizer(s) against *L. monocytogenes*, and to further seek to verify their efficacy on multiple apple packing lines. The proposed project will develop information for apple producers about the practical efficacy of antimicrobial interventions under commercial packing conditions. Also, there is a high potential for transferability to other tree fruit and fruit vegetable commodities with similar surface traits and postharvest handling systems.

OBJECTIVES

Objective 1: Validate the efficacy of selected sanitizers against *L. monocytogenes* on whole apple surfaces through laboratory testing.

Objective 2: Verify the selected sanitizer interventions on model/pilot packing line and representative commercial apple packing lines in two states.

METHODS

Work to date has mainly focused on Objective 1, to validate the efficacy of selected sanitizers against *L. monocytogenes* on whole apple surfaces. Unwashed and non-waxed apples of selected varieties (Granny Smith and Fuji) were individually and separately inoculated to establish 1×10^6 CFU/apple of a 3-strain cocktail of *L. monocytogenes* using dip inoculation. Inoculated apples were held at room temperature for 48h, then subjected to different sanitizer treatments at selected concentrations for different durations (15, 30 and 120 seconds), and water was used as a control. Bacterial inoculation levels were enumerated right after inoculation and 48h post-inoculation. Microbial survival, as log-reduction on inoculated apples, was analyzed per our standard methods. The survival of *L. monocytogenes* in sanitizer solutions was further analyzed using a membrane filtration method.

Table 1. Survival of *L. monocytogenes* in sanitizer solutions after inoculated Granny Smith apple wash.

	Sanitizer contact time		
	15 s	30 s	2 min
Water	$(0.96 \pm 0.01) \times 10^{4*}$	$(1.48 \pm 0.02) \times 10^4$	$(2.01 \pm 0.01) \times 10^4$
Chlorine	ND	ND	ND
0.125% JC	1.01 ± 0.02	0.69 ± 0.02	0.42 ± 0.02
0.25% JC	0.63 ± 0.01	0.40 ± 0.02	0.19 ± 0.01
0.5% JC	0.35 ± 0.02	0.11 ± 0.02	ND
1.0% JC	0.23 ± 0.01	0.07 ± 0.02	ND

*: Mean ± SEM, n=12. CFU/ml. ND: not detected

RESULTS TO DATE

We have established the inoculation method and evaluated the antimicrobial efficacy of chlorine and sanitizer JC9450 against *L. monocytogenes* on Fuji and Granny Smith (GS) apples for different contact durations. 100ppm chlorine at pH6.8 had a limited antimicrobial efficacy against *L. monocytogenes* (~1 log reduction) even at a 2-minute contact time for both Fuji and GS apples. At a 2-minute contact time, 0.5-1.0% JC9450 reduced *L. monocytogenes* established on apples by 3.5-4.5 log. JC9450 was slightly more effective on Fuji apples (Figure 1). In general, the antimicrobial efficacy of JC9450 decreased with reduced contact time (Figure 2). 0.5% (v/v) JC9450 at 15s and 30s of contact time caused 1.5 and 2.5 log reduction of *L. monocytogenes* on GS apples, while 1.0% JC9450 wash for 30s and 15s resulted in 2.5 and 3.5 log reductions, respectively (Figure 2). Survival of *L. monocytogenes* in spent 100ppm chlorine and 0.5% and 1.0% sanitizer solution was under the detection limits (Table 1). We are current testing and will test the antimicrobial efficacy of peracetic acid, neural electrolyzed water, chlorine dioxide and other sanitizers. In addition, we are conducting a formal survey to determine the range of spray manifold set up and utilization in the apple industry. To date, we have found that water temperature, nozzle type, and general spray bar arrangement are similar, while water pressure, dwell time of fruit under the system, and type of sanitizer vary greatly between packing lines.

BENEFITS TO THE INDUSTRY

The proposed project is expected to develop information for apple producers about the practical efficacy of antimicrobial interventions under commercial packing conditions. The outcomes of this project will provide a foundation for validated process controls, verification of standard operating procedures, and monitoring protocols which will be accessible to the apple industry to support compliance with FSMA Preventive Controls requirements and customer required audit schemes. Methods developed will not only be applicable to *L. monocytogenes* safety but will also likely be useful in controlling other foodborne pathogens. Such methods should be easily transferred to the other sectors of tree fruit and fruit vegetable commodities with similar surface traits and postharvest handling systems.

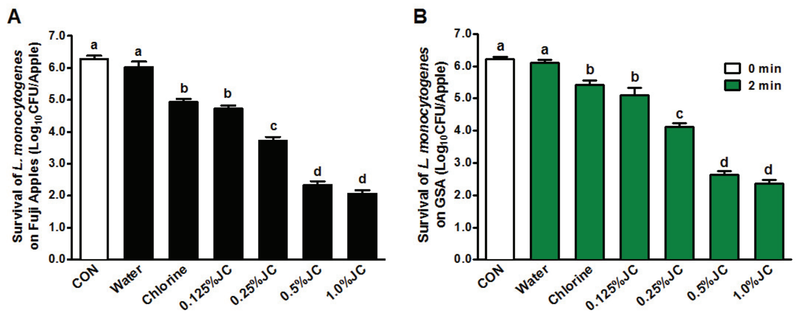

Figure 1. Antimicrobial efficacy of chlorine and JC9450 against *L. monocytogenes* established on Fuji (A) and GS apples (B) apples. CON: *L. monocytogenes* counts on apple before sanitizer intervention; Water: inoculated apples were washed with water for 2 min; Chlorine: 100ppm, pH6.8; JC: JC9450 applied at concentration of 0.125% - 1.0% (v/v). Mean ± SEM, n=12. Histogram bars with the same letter do not differ significantly at a P value of 0.05.

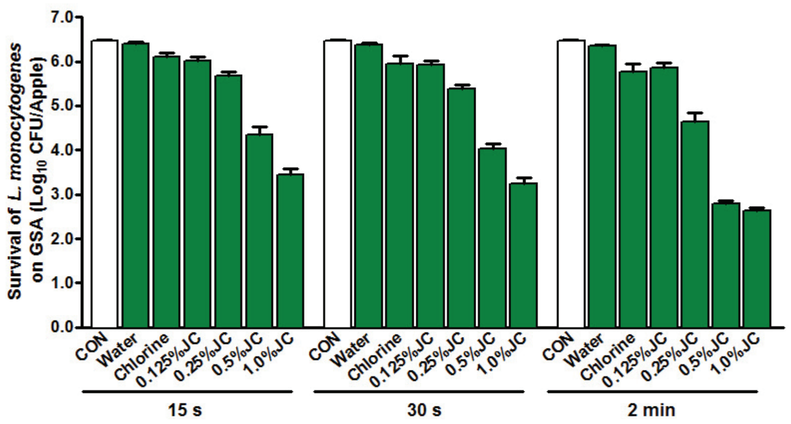

Figure 2. Antimicrobial efficacy of chlorine and JC9450 against *L. monocytogenes* established on GS apples at contact time of 15s, 30s and 2 min. CON: *L. monocytogenes* counts on apple before sanitizer intervention; Water: inoculated apples were washed with water for 2 min; Chlorine: 100ppm, pH6.8; JC: JC9450 applied at concentration of 0.125% - 1.0% (v/v). Mean ± SEM, n=12.

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ACKNOWLEDGEMENTS

We thank Allan Brothers Inc and Stemilt Growers LLC for their generous donation of fresh apples, and Pace International and Jenfitch Inc for their generous donation of sanitizers. We thank Lina Sheng and a team of dedicated graduates and staff from WSU and UC Davis for their contributions.

LENGTH OF FUNDING

January 1, 2017 – December 31, 2018

CENTER for PRODUCE SAFETY