RESEARCH IN PROCESS

# Preservation of stone fruits by spray application of edible coatings with antimicrobial properties


## SUMMARY

Results have shown that surface roughness can play a role in potential formation of a biofilm produced under laboratory conditions using *Listeria monocytogenes* Scott A. Optimization of biofilm formation conditions is underway, and media experiments have been completed. We determined that HTM (Hsiang-Ning-Tsai) broth supports higher overall biofilm mass compared with Modified Welshimer's broth (MWB). Testing of coating solutions showed that incorporation of bacteriophage P100 into Aloe vera or whey protein isolate provides significantly higher zones of inhibition in spot-on-lawn assays compared with sodium alginate, methyl cellulose and controls.


## OBJECTIVES

1. Develop a flow-through system to determine cleaning efficacy on surfaces with flat and topographical features and determine surface role and cleaning procedure in the possible pathogen contamination of stone fruits.
2. Evaluate novel fruit coating formulations with antimicrobial properties that can be developed as brush-independent (spray) applications and can replace traditional wax treatments to maintain fruit integrity and shelf life.
3. Determine efficacy and properties of selected coating formulations in challenge studies in controlled conditions.

## METHODS

**Surface roughness of rollers:**

- Peach roller samples cut into identical coupons
- Surface roughness characterized, sterilized and used to grow *L. monocytogenes* biofilms on surfaces
- *L. monocytogenes* Scott A transformed with plasmid pNF8 to constitutively express green fluorescent protein (GFP)
- Biofilms grown in shallow containers; 1/10 diluted tryptic soy broth supplemented with erythromycin for plasmid maintenance

**Comparison of chemically-defined minimal media:**

- Modified Welshimer's broth (MWB) and Hsiang-Ning-Tsai broth (HTM)
- Biofilms grown up to 72 hours at 25°C; fresh media added to individual wells
- Biofilms analyzed at 0, 12, 24, 36, 48, 60 and 72 hours; n=3

**Coating formulations for phage incorporation:**

- Peach coating formulations produced with and without Listex™ P100 phage mixture
- Coating solutions: methylcellulose, Aloe vera, whey protein isolate, and sodium alginate
- Phage concentration of $2 \times 10^8$ PFU/mL (PFU = plaque forming units) in each solution
- Spot-on-lawn assays: tryptic soy agar seeded with 8 log (CFU/mL) of *L. monocytogenes* Scott A

## RESULTS TO DATE

**Confocal microscopy of polymeric surfaces** (see Figure 1)**:**

(A) Used material with high porosity (avg surface roughness 3.531 ± 0.112 microns)
(B) New material with low porosity (avg surface roughness 1.305 ± 0.231 microns)
(C) Biofilms of *L. monocytogenes* Scott A grown on porous (used) panels C1 and C2, and new (smooth) panel C3

**Comparisons between the two minimal media:**

Comparisons between the minimal media revealed that MWB supported L. monocytogenes growth at higher densities in the bulk phase (7.33 log CFU/ml) than did HTM (6.12 CFU/ml).

The overall biofilm biomass obtained by crystal violet staining was higher for HTM (optical density at 595 nm of de-stained biofilm, normalized value 4.79 ± 0.13) compared with MWB (optical density at 595 nm of de-stained biofilm, normalized value 3.34 ± 0.11).

## BENEFITS TO THE INDUSTRY

- In this project, we hypothesize that *L. monocytogenes* survival on stone fruits can be inhibited by a coating exhibiting both antimicrobial and preservation properties.
- The overall aim of the project is to characterize the conditions favorable for *L. monocytogenes* contamination of stone fruit in the packinghouse, and design measures to reduce pathogen survival and avoid cross-contamination.

Figure 1.

(A)

(B)

(C)

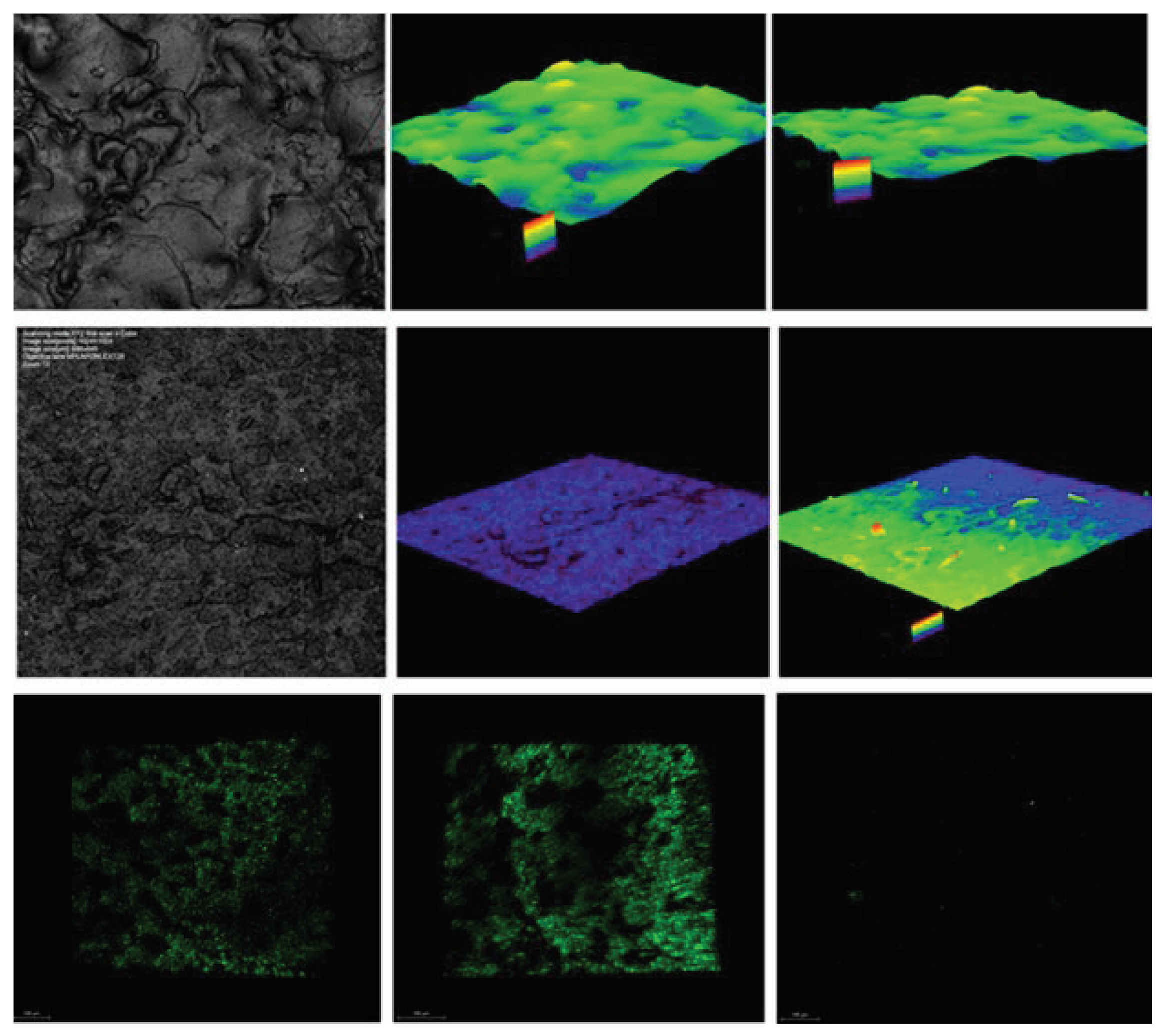
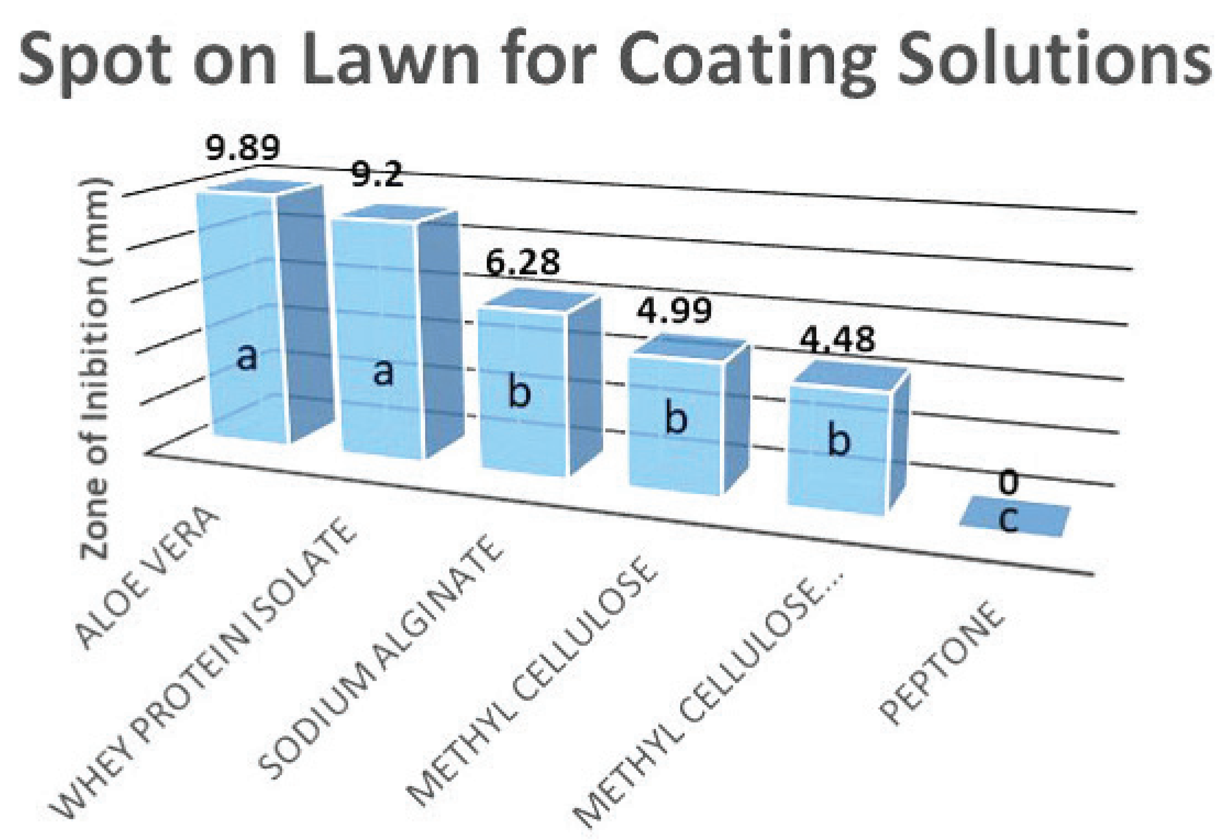


Figure 2.





## CONTACT

Kay Cooksey, Ph.D.
Clemson University
E: kcookse@clemson.edu



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## AUTHORS

Kay Cooksey, Claudia Ionita (Co-PI), S. Kessler and R. Ramey



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