

# Development of a novel microbicide for control of foodborne pathogens

## Introduction

The efficacy of many chlorine-based sanitizers currently used in food processing facilities is reduced when organic matter is present, whereby their usefulness as an antimicrobial is mitigated. Effective sanitizers that are effective, practical, cost-efficient and environmentally-friendly are needed to control food borne pathogens and their biofilms in food processing facilities. To gain all these purposes and at the same time to ensure the quality of treated food unchanged, various organic acids and detergents were evaluated individually and in combination for their bactericidal activity. We revealed that either organic acids or detergents did not show significant bactericidal effect when used individually. However, a combination just including two chemicals, levulinic acid and sodium dodecyl sulfate (SDS) can inactivate all bacteria as tested. Results revealed that a solution with 0.5% levulinic acid and 0.05% SDS provided a ca. 7logCFU/ml reduction of *E. coli* O157:H7, *S. Enteritidis*, and *S. Typhimurium* DT104 within 1 min (processing time). Its effect as a rinse solution to remove *E. coli* O157:H7 and Salmonella was confirmed with romaine lettuce and poultry skin.<sup>1</sup> The advantage of levulinic acid over other organic acids is its characteristics; including it does not produce corrosion, its safety to human, and it can keep the quality of treated produce.

The number of sprout-related outbreaks has an increased tendency. The method to kill all the pathogens (human and plant) in seeds and to guarantee their germination rate is not interfered is demanded. Studies were done to determine the best concentration and exposure time for treatment of the alfalfa seeds with levulinic acid plus SDS to inactivate *E. coli* O157:H7 and Salmonella and not adversely affect seed germination. Alfalfa seeds contaminated with *E. coli* O157:H7 were subsequently dried at 21°C in a laminar flow hood for up to 72h. Results demonstrated that a 5-min treatment at 21°C of alfalfa seeds contaminated with 108 *E. coli* O157:H7 or *S. Typhimurium* DT 104 of a solution containing 0.5% levulinic acid plus 0.05% SDS reduced *E. coli* O157:H7 and *S. Typhimurium* DT 104 populations by 5.6 and 6.4log10CFU/g, respectively. Treatment of seeds contaminated with 104 *E. coli* O157:H7 or *S. Typhimurium* CFU in a glass beaker and then in a stomacher bag with 0.5% levulinic acid plus 0.05% SDS at 21°C for a total of 20min reduced *E. coli* O157:H7 or *S. Typhimurium* DT 104 in all samples (25g) to undetectable levels by a direct plating method (<0.7log10CFU/g), but 8 of 10 samples were detectable by selective enrichment culture. Germination rates of alfalfa seeds treated with 0.5% levulinic acid plus 0.05% SDS for up to 1h at 21°C were compared with a treatment of 20mg calcium hypochlorite/ml and tap water only treatment. Results revealed that treatment with 0.5% levulinic acid plus 0.05% SDS for 1h at 21°C did not adversely affect alfalfa seed germination compared to the control treatment with tap water, whereas treatment with 20mg calcium hypochlorite for 1h substantially reduced germination.<sup>2</sup> Currently their efficacy to kill all the plant pathogens is in progress. Many food borne outbreaks were closely linked with the biofilm formation in food processing facilities. The antimicrobial efficacy of this sanitizer applied either as a liquid or as foam (under pressure) demonstrated excellent bio film removal specificity when compared with other sanitizers.<sup>3,4</sup> Contaminated

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Tong Zhao

Center for Food Safety, University of Georgia, Georgia

**Correspondence:** Tong Zhao, Center for Food Safety, University of Georgia, Georgia 30223, Email tongzhao@uga.edu**Received:** April 2, 2015 | **Published:** May 12, 2015

slicer surfaces sprayed with this sanitizer as a foam (45-55psi) reduced within 1min 6.0 to 8.0log CFU of the three pathogens per blade. Results revealed that pathogenic transfer rate between slicers and foods depend on food contact locations on slicers, the composition of food, and the nature of pathogens. Also, Levulinic-based sanitizer applied as foam can be used as a potential method to remove microbial contamination on the surface of deli slicers.<sup>5</sup>

The safety of levulinic acid were tested for humans and thoroughly evaluated by world health organization because its addition in tobacco and it has GRAS status for direct addition to food as a flavoring substance or adjunct (FDA 2008, 21 CFR, 172.515). We confirmed its property by soaking whole Romaine lettuce in 0.5% levulinic acid plus 0.05% SDS for either 15 or 30min, then rinsing the lettuce with water three times and storing the treated lettuce and lettuce rinsed with water only (control) at 5°C for up to 14days to observe the color change. There were no visual differences between the lettuce treated with 0.5% levulinic acid plus 0.05% SDS for 15 or 30min and the lettuce rinsed with water only. Sodium dodecyl sulfate has GRAS status for multipurpose additives (FDA 2007, 21 CFR, 172.822). Its safety has been demonstrated by dental industry. Since our first publication with development of this sanitizer, many researchers have validated its application in different field. Dental doctors use it as a mouth rinse to remove dental biofilms in vitro and in vivo. Their studies revealed its efficacy to remove dental biofilm is significantly better than the commonly used Listerine.<sup>6</sup> Its efficacy as virocidic to inactivate nor virus and influenza A virus in various foods has been validated by different researchers<sup>7,8</sup> at present its application in pre-harvested produce for inactivation of food borne pathogens and plant pathogens has been documented.

## Conclusion

An alternative to chlorine-based sanitizer is developed and validated for its efficacy in various applications at food processing facilities for reduction of food borne pathogens and for their biofilm removal.<sup>9,10</sup> At present a patent for its application was issued by U.S. patent office and licensed by Health Pro Inc. Its efficacy has been validated in various applications.

## Acknowledgements

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## Conflict of interest

The author declares no conflict of interest.

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