

Title:	Innovative Technologies and Process Optimization for Food Safety Risk Reduction Associated with Fresh and Fresh-cut Leafy Green Vegetables		
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Non-Technical Summary

Food-borne illness outbreaks associated with leafy green vegetables have severely impacted public health, consumer confidence, the produce industry's economic well-being, and attainment of national nutritional goals. Currently, no processing technology eliminates human pathogens without compromising quality. Industry critically needs tools to reduce pathogen levels and prevent cross-contamination during fresh-cut processing, and prevent pathogen proliferation in the supply chain. This project addresses these needs using a systems-based, action-driven, multiple-hurdle approach. Our trans-disciplinary project comprises five main objectives: 1) optimize produce wash systems to improve sanitizer efficacy and prevent cross-contamination during washing and cutting; 2) develop innovative washing processes using ultrasound, surfactants, and sanitizers to more effectively inactivate pathogens; 3) reduce pathogen proliferation by improving retail cold display; 4) disseminate information to end users and facilitate technology adoption; and 5) evaluate economic, social, and environmental impacts, including reduced water, energy, and chlorine consumption. This project uses an integrated system-based approach to fresh-cut processing and retail display, considering both food safety and quality. Key features include pathogen inoculation to emulate realistic field contamination, a dedicated semi-commercial pilot plant simulating commercial fresh-cut washing/cutting, and new retail cold-display technology. Industry support is evident through active input in proposal development, and significant in-kind commitments, including commercial-facility access. Ongoing industry consultation will ensure practical and cost-effective solutions, hastening adoption. Expected significant reductions in pathogen contamination will reduce food-borne illness, restore consumer confidence in leafy greens, promote sustained industry growth, and, in the long term, improve public health by increasing fresh produce consumption.

Accomplishments

Major goals of the project

The overall goal of this project is to reduce the risk of pathogen contamination on fresh-cut leafy greens. This trans-disciplinary project comprises five main objectives: 1) optimize produce wash systems to improve sanitizer efficacy and prevent cross-contamination during washing and cutting; 2) develop innovative washing processes using ultrasound, surfactants, and sanitizers to more effectively inactivate pathogens; 3) reduce pathogen proliferation by improving retail cold display; 4) disseminate information to end users and facilitate technology adoption; and 5) evaluate economic, social, and environmental impacts, including reduced water, energy, and chlorine consumption.

What was accomplished under these goals?

IMPACT

Our research has provided FDA, leafy green processors, and food retailers the scientific basis for landmark changes in standards and operations to prevent pathogen cross-contamination, spread, and proliferation during fresh-cut produce washing and retail display.

1. Our groundbreaking research is used by FDA and industry to reset food safety standards. We are the first to show that the industry-standard "Control Limit" chlorine concentration does not prevent pathogen cross-contamination, and that re-washing of contaminated product is an ineffective "Corrective Action" to rectify process failures. This seminal work overturned historical industry practices by documenting the risks associated with operating practices previously considered safe. We also determined the necessary and sufficient sanitizer concentration to prevent pathogen cross-contamination and spread. These results are now incorporated into an interagency and industry task force document supporting Food Safety Modernization Act implementation. Canadian researchers have used our findings to develop mathematical models to predict contamination, and the US Department of Homeland Security has incorporated our results into anti-terrorism programs.

2. Established the scientific basis for fundamentally new approaches to improve process controls for organic load and sanitizers in produce washing. By delineating interactions between free-chlorine concentration, organic load, chlorine demand, produce quality, and safety, we, in partnership with fresh-cut processors, demonstrated the direct effect of chlorine concentration on pathogen cross-contamination, and deleterious effects of organic load on free-chlorine levels and pathogen survival. These findings identified key factors in bacterial inactivation during commercial washing, which helped industry set priorities, enabled development of science-based food safety practices, operational standards, and process controls. Moreover, these results have stimulated development of innovative, processing equipment designs to reduce organic loads and improve wash-water quality.

3. Invented four novel technologies to overcome roadblocks in fresh produce wash research and operations: 1) a novel microfluidic mixer to determine sub-second time-dose responses for bacterial inactivation by anti-microbial agents. Currently in use by the CDC and University of Maryland, this patent-pending technology filled an instrumentation void, allowing researchers to accurately determine anti-bacterial kinetics; 2) a novel in-flight washer which removes organic exudate from fresh-cut produce immediately after cutting, reducing loss of sanitizer strength, and water and chemical consumption; 3) a chlorine-dosing program enabled the development and transformation of chlorine control from currently feedback to feed forward, and eliminate trial-and-error practices; and 4) biomimetic plant surfaces to facilitate reproducible experiments involving microbial disinfection and attachment/release from plant surfaces, and eliminating leaf-to-leaf variability in experiments with real produce.

4. Identified a cost-effective mechanism to improve cold chain integrity and fully implement food safety preventive controls during retail display. Open refrigerated display cases typically have significant temperature nonuniformity, presenting technical challenges for maintaining temperature below 5 °C at the front, without freezing damage at the rear. Our research found that retrofitting open cases with doors achieved Food Code compliance, with nearly-uniform product temperatures below 5 °C throughout the case. These conditions prevented growth of all major human pathogens and improved product quality and shelf life. Moreover, energy costs were 69% less than for open cases, allowing retrofit cost recovery in less than two years. Reduced costs for product rotation and savings from reduced spoilage are also expected. We informed retailers of these results via meetings, webinars, publications, and personal contact; use of doors on fresh-cut produce retail cases has substantially increased in the last two years.

Output

Obj. 1 Optimize fresh-cut produce wash system configurations and operations. 1) Investigated pathogen survival/inactivation dynamics during chlorine depletion/replenishment, demonstrating that no pathogens survive when free-chlorine is maintained above a critical level, irrespective of organic loading; 2) Quantified effects of flow on shearing off of pathogen colonies from spinach surfaces (in collaboration with Massachusetts Institute of Technology); 3) Identified major organic components that deplete free chlorine in wash water, thus providing specific targets for removal in treatment of spent process water for reuse (in collaboration with Johns Hopkins Univ.); 4) Invented four novel technologies to overcome mechanistic roadblocks in fresh produce wash research and operations; 5) Demonstrated that incorporating a washing step prior to cutting significantly improves pathogen inactivation and prevention of cross-contamination.

Obj. 2 Develop innovative approaches, processes, and hardware to wash leafy greens. The Univ. of Illinois Urbana-Champaign (UIUC) team tested combinations of sonication, sanitizers, and surfactants on pathogen reduction and produce quality. This work significantly advanced understanding of ultrasonic pathogen inactivation, and optimized process parameters

to improve inactivation while maintaining quality. Separately, the UIUC team further verified that washing-before-cutting provides significant benefit in reducing microbial counts and improving sanitizer efficacy.

Obj. 3 Develop/test technology to improve cold-chain integrity and compliance with FDA temperature control regulations for produce safety. We addressed the large temperature variations of bagged salad displayed in open refrigerated display cases, in the front often above the FDA Food Code's 5 °C limit. We evaluated many options, including optimizing operating parameters, using insulators and phase change materials, installing curtains, and retrofitting open cases with clear glass doors. For typical display cases, we mapped temperature distributions, and conducted quality and safety studies. After selecting retrofitting with glass doors, we conducted large validation trials with several leafy greens, to assess freshness, visual quality, human pathogen growth, and energy consumption. We showed that installing clear doors achieved nearly uniform product temperature (0.6 to 4.2 °C) and Food Code compliance, and prevented pathogen growth. Product quality and shelf-life improved, and 69% less energy was consumed for typical door opening frequencies and durations (every 10 min for 6 sec).

Obj. 4 Provide outreach and facilitate technology transfer. Detailed in "How have the results been disseminated to communities of interest" section.

Obj. 5 Evaluate social, economic, and environmental impacts of implementation of these technologies. Economists (Univ. Arizona and USDA-ERS) interviewed five major fresh-cut processors to analyze costs/benefits of ultrasound in postharvest washing of leafy greens. Key factors affecting adoption were identified and shared with industry and other researchers. Retrofitting doors to display cases in actual retail settings was also analyzed. For an anonymous regional supermarket chain (120 stores), same-store sales before and after retrofitting, and between stores with and without retrofitting, were compared. No negative sales impact of door installation was found. The results should promote retail display of bagged salad behind glass doors, given the benefits for food safety, quality, and energy savings.

What opportunities for training and professional development has the project provided?

Training/professional development was provided to eight undergraduate and three graduate students from University Maryland, and one graduate student each at Johns Hopkins University, MIT, UIUC, and University of Arizona. Also trained five postdoctoral associates and four visiting professors at USDA-ARS, and one postdoctoral associate each at Johns Hopkins University and MIT. Graduate students and postdoctoral associates attended professional meetings, symposia, and workshops organized by the Institute of Food Technologists, International Association for Food Protection, and the National Institute for Mathematical and Biological Synthesis, and Center for Produce Safety. Project also provided training opportunities to minority students and those from Hispanic Serving Institute, and Historically Black College.

How have the results been disseminated to communities of interest?

A multitude of mechanisms has been used to communicate the findings to stakeholders in industry, academia, and policy making bodies. These include publications in peer-reviewed journals and trade publications, presentations at scientific and industry meetings, face-to-face meetings and social media outreach, on-site visits to stakeholders and hosts of stakeholder visits to our research facilities, and working side-by-side in research and doing demonstrations at stakeholders' pilot plant and commercial processing facilities. Outreach activities were carried out by researchers and extension specialists located in major specialty crop production and processing areas.

- Organized and hosted two food safety conferences and stakeholder visits at Beltsville, Md., in conjunction with the produce industry public policy conferences held in Washington, D.C. Over 80 people, including technical executives from industry, FDA policy specialists, researchers, students, and industry journalists, attended these meetings. All project team members presented research findings and explained, in nontechnical language, how to translate these findings into action steps.
- Informed stakeholder advisory board members about our new findings and sought their input during two face-to-face meetings, several video conference calls, and many email exchanges.
- The PD served on the produce industry association's food safety and technology council and shared research findings with the industry technical executives during the council meetings (often attended by more than 50 companies) held twice a year.
- Co-organized, in collaboration with the NSF-supported National Institute for Mathematical and Biological Synthesis, the first international workshop on "Modeling Microbial Contamination of Fresh Produce".
- Fully utilized Internet video outreach and social media interfacing to disseminate information to stakeholders. The University of Arizona team developed and maintained a website (<https://www.cals.arizona.edu/fps/node/58>) that updated project activities and research publications. We also used Facebook, Twitter, and Pinterest for outreach activities. Since the live release in 2013, these sites have generated over 11,170 global interactions in fresh produce safety, and continue to grow daily.
- Created and published a YouTube channel with a collection of 4 targeted training and demonstration modules, providing project impacts directly to users. Total viewership exceeded 500 from nine countries within the two months after its launch. Video modules can be viewed on the Fresh Produce Safety Channel (<http://www.youtube.com/user/FreshProduceSafety/featured>).

• Also held five webinars with produce industry and government regulatory personnel, reaching more than 40 companies in the produce processing and retailing industries.

What do you plan to do during the next reporting period to accomplish the goals?

{Nothing to report}

Participants

Actual FTE's for this Reporting Period

Role	Non-Students or faculty	Students with Staffing Roles			Computed Total by Role
		Undergraduate	Graduate	Post-Doctorate	
Scientist	2.4	0	0	5	7.4
Professional	1.2	0	0	0	1.2
Technical	1.2	0	1.2	0	2.4
Administrative	2.5	0	0	0	2.5
Other	0.5	3.2	0	0	3.7
Computed Total	7.8	3.2	1.2	5	17.2

Student Count by Classification of Instructional Programs (CIP) Code

Undergraduate	Graduate	Post-Doctorate	CIP Code
2	2	1	01.04 Agricultural and Food Products Processing.
1	1	1	14.12 Engineering Physics.
7		2	01.10 Food Science and Technology.
2		1	26.01 Biology, General.

Target Audience

Fresh and fresh-cut produce processors, packers, retailers; Food service QA manager; Food scientists; Food display equipment manufacturers; Food safety managers, FDA Consumer Officer; and CDC researchers.

In addition, project team provided trainings and laboratory experience to students of diverse ethnicity, including Hispanic and African-American student. We provided on-site visit to Hispanic Serving Institution (HSI) program directors and mentored HSI and African-American students.

Products

Type	Status	Year Published	NIFA Support Acknowledged
Journal Articles	Published	2015	YES

Citation

De Frías, J.A., Luo, Y., Kou, L., Zhou, B. and Wang, Q. 2015. Improving spinach quality and reducing energy costs by retrofitting retail open refrigerated cases with doors. *Postharvest Biology and Technology*. 110:114-120.

Type	Status	Year Published	NIFA Support Acknowledged
Journal Articles	Published	2015	YES

Citation

Lin, B., Luo, Y., Zhang, Z., Zhang, B., Zhou, B., and Wang, Q. 2015. Development of silver/titanium dioxide/chitosan adipate nanocomposite as an antibacterial coating for fruit storage. *LWT. Food Science and Technology*. 63:1206-1213.

Type	Status	Year Published	NIFA Support Acknowledged
Journal Articles	Published	2015	YES

Citation

Zhou, B., Luo, Y., Nou, X., Lyu, S., and Wang, Q. 2015. Survival dynamics of Salmonella enterica, Listeria monocytogenes, and Escherichia coli in wash water during simulated chlorine depletion and replenishment processes. Food Microbiology. 50:88-96.

Type	Status	Year Published	NIFA Support Acknowledged
Journal Articles	Published	2015	YES

Citation

Xiao Z, Bauchan G, Nichols-Russell L, Luo Y, Wang Q, Nou X. 2015. Proliferation of Escherichia coli O157:H7 in soil and hydroponic microgreen production systems. Journal of Food Protection. 78:1785-1790.

Type	Status	Year Published	NIFA Support Acknowledged
Other	Published	2015	YES

Citation

De Frías, J.A., Luo, Y., Zhou, B., Millner, P., and Nou, X. 2015. Lower temperatures, in cases with doors, improve produce quality and safety with reduced energy consumption. Produce Business Magazine. 31:14.

Type	Status	Year Published	NIFA Support Acknowledged
Journal Articles	Published	2015	YES

Citation

Yan, S., Luo, Y., and Yang, T. 2015. The mechanism of ethanol treatment on inhibiting lettuce enzymatic browning and microbial growth. LWT - Food Science and Technology. 63:383-390.

Type	Status	Year Published	NIFA Support Acknowledged
Journal Articles	Awaiting Publication	2016	YES

Citation

Weng, S.C., Luo, Y., Li, J., Zhou, B., Jacangelo, J., and Schwab, K.J. 2016. Vegetable wash water chemical characterization and chlorination. Food Control. 60:543-551.

Type	Status	Year Published	NIFA Support Acknowledged
Journal Articles	Published	2015	YES

Citation

Zhang, B., Luo, Y., Zhou, B., Wang, Q., and Millner, P.D. 2015. A novel microfluidic mixer-based approach for determining inactivation kinetics of Escherichia coli O157:H7 in free chlorine solutions. Food Microbiology. 49: 152-160.

Type	Status	Year Published	NIFA Support Acknowledged
Journal Articles	Published	2015	YES

Citation

Huang, J., Luo, Y., and Nou, X. 2015. Growth of Salmonella enterica and Listeria Monocytogenes on fresh-cut cantaloupe under different temperature abuse scenarios. Journal of Food Protection. 78:1125-1131.

Type	Status	Year Published	NIFA Support Acknowledged
Conference Papers and	Published	2014	YES

Citation

Wilson, R., Bevington, R., Nolte, K.D., and Ravishankar, S. 2014. Extending Research Impacts to a General Audience Via Video: The Good, The Bad, And The Ugly. Abstract published in the proceedings at the 2014 American Society for Horticultural Science Annual Meeting, Orlando, FL.

Type	Status	Year Published	NIFA Support Acknowledged
Conference Papers and	Published	2014	YES

Citation

Bevington, R., Nolte, K.D. and Ravishankar, R. 2014. Best Methods to Present Research Information via Video Formats to a Public Audience. Abstract published in the proceedings at the 2014 American Society for Horticultural Science Annual Meeting, Orlando, FL.

Type	Status	Year Published	NIFA Support Acknowledged
Conference Papers and	Published	2013	YES

Citation

Bevington, R., Ravishankar, S. and Nolte, K.D. 2013. How To: Making an Informative Video from Start to Finish. Abstract published in the proceedings at the 2013 American Society for Horticultural Science Annual Meeting, Palm Desert, CA.

Type	Status	Year Published	NIFA Support Acknowledged
Conference Papers and	Published	2015	YES

Citation

Zhang, B., Luo, Y., Pearlstein, A., Bauchan, G., Nou, X., Wang Q., Millner, P. 2015. Fabrication of Biomimetically Patterned Surfaces and Their Application to Probing Plant-Bacteria Interactions at the 2015 Institute of Food Technologists Annual Meeting, Chicago, IL, July 2015.

Type	Status	Year Published	NIFA Support Acknowledged
Conference Papers and	Published	2015	YES

Citation

Zhang, B., Luo, Y., Millner, P. 2015. A Novel Microfluidic Mixer-Based Approach for Determining Subsecond Inactivation Kinetics of Escherichia Coli O157:H7 to Prevent Cross-Contamination at the 2015 Institute of Food Technologists Annual Meeting, Chicago, IL, July 2015.

Type	Status	Year Published	NIFA Support Acknowledged
Conference Papers and	Published	2015	YES

Citation

De Frias, A., Luo, Y., Kou, L., Zhou, B. 2015. Retrofitting Open Refrigerated Display Cases with Doors for Packaged Greens: Greater Compliance with FDA Food Code and Improved Food Quality at the 2015 Institute of Food Technologists Annual Meeting, Chicago, IL, July 2015.

Type	Status	Year Published	NIFA Support Acknowledged
Conference Papers and	Published	2015	YES

Citation

Zhou, B., Luo, Y., De Frias, A., Nou, X., Millner, P., Wang, Q. 2015. Fate of human pathogens in packaged leafy greens stored in a refrigerated display cabinet with doors at the 2015 Institute of Food Technologists Annual Meeting, Chicago, IL, July 2015.

Patent(s) and Plant Variety Protection(s)

Application Number	Application Filing Date	Title
62/048,507	09/10/2014	Micro-fluidic mixer and method of determining pathogen inactivation via antimicrobial solutions
Docket No. 0161.15	09/21/2015	Device and method for the immediate removal of organic exudate from fresh-cut produce by contactless in-flight washing

Other Products**Product Type**

Audio or Video

Description

Developed 4 targeted training and demonstration YouTube video modules, providing project impacts directly to users. Videos can be viewed on the Fresh Produce Safety Channel (<http://www.youtube.com/user/FreshProduceSafety/featured>).

Product Type

Other

Description

Developed and maintained a website (<https://www.cals.arizona.edu/fps/node/58>) that updated project activities and research publications.

Changes/Problems

{Nothing to report}