Integration of Multiple Interventions to Enhance Microbial Safety, Quality, and Shelf-life of Foods

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Location: *RCPM, WYNDMOOR, PA*

ARS 2016-2020 Action Plan
Integration of Multiple Interventions Project

Addresses two official ARS Research Priorities

Develop / refine / integrate effective interventions

Improve Safety/quality/shelf life

Specific Objective

Develop and optimize single intervention technologies to reduce pathogen populations, maintain quality, and extend shelf-life of foods

Determine the synergistic/additive effectiveness of combining non-thermal processing, effective sanitizer treatment, antimicrobial coating/packaging

Current practice

- Currently chlorine based washing is widely used in the industry
- Effect of chlorine wash on pathogens are inconsistent and chlorine washing raised some serious public health concern
- Need for development of alternative intervention technologies that are safe and effective to minimize the risk of food borne illness

Single nonthermal intervention technologies to reduce pathogen populations

- Light Technology
  - -Static Light (254 nm)
  - -Pulsed Light (200-1100 nm)
- Ultra low dose Ionizing radiation
- Antimicrobial treatment
  - - Organic acids, chemical sanitizers, natural antimicrobials
  - - Novel antimicrobial formulation : HEN, LAPEN and more under development in joint work with collaborator

- High Pressure Processing: 200 - 500 MPa at 8-15°C for 2-5 min
- Aerosolized antimicrobials treatment: H_2O_2, AcOH, New formulations

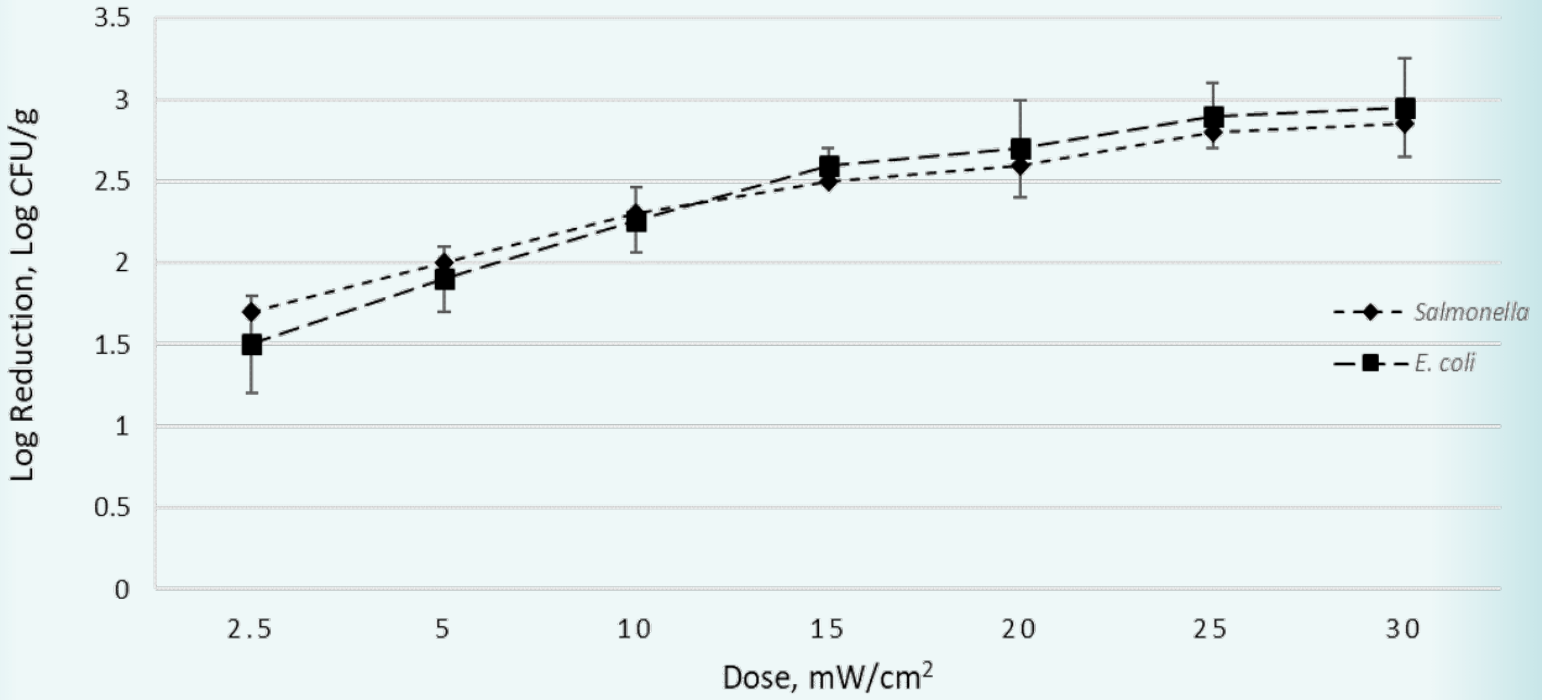
Single intervention technologies to reduce pathogen populations, maintain quality, and extend shelf-life

The effects of different UV-C dose treatments on inactivation of *S. enterica* and *E. coli* O157:H7 on whole grape tomato surface and stem scar

Dose ($kJ\ m^{-2}$)	*E. coli* O157:H7 population reduction ($log\ CFU\ tomato^{-1}$)		*Salmonella enterica* population reduction ($log\ CFU\ tomato^{-1}$)	
	Surface	Stem scar	Surface	Stem scar
0.6	2.25 ± 0.11 Da	1.60 ± 0.10 Eb	2.15 ± 0.23 Da	1.93 ± 0.11 Da
1.2	2.70 ± 0.08 Ca	2.27 ± 0.09 Db	2.33 ± 0.16 CDa	2.16 ± 0.08 CDa
2.4	3.05 ± 0.16 Ba	2.59 ± 0.10 Cb	2.57 ± 0.03 BCa	2.37 ± 0.09 BCa
3.6	3.29 ± 0.10 ABa	2.83 ± 0.12 BCb	2.75 ± 0.11 ABa	2.56 ± 0.11 ABa
4.8	3.44 ± 0.22 Aa	3.04 ± 0.08 ABb	2.90 ± 0.25 ABa	2.67 ± 0.07 ABa
6.0	3.49 ± 0.21 Aa	3.17 ± 0.07 Ab	3.06 ± 0.39 Aa	2.81 ± 0.06 Aa

Mukhopadhyay et al., Food Control 44:110-117, 2014

Inactivation of Pathogens by UV-C
Salmonella on tomato and E. coli O157:H7 on leafy green surface



Mukhopadhyay et al., Food Control 44:110-117, 2014

Single intervention technologies (contd.)

Dose effect on TAB population during storage at 5 C for 3 weeks

Storage time, day	Control	UV-C treated, dose, kJ/m^2				
		0.6	1.2	2.4	4.8	6.0
0	$4.57 \pm 0.39a^{B}$	$4.54 \pm 0.37a^{B}$	$4.40 \pm 0.62a^{C}$	$4.10 \pm 0.59a^{B}$	$4.12 \pm 0.60a^{B}$	$3.92 \pm 0.24a^{C}$
7	$6.07 \pm 0.63a^{A}$	$5.76 \pm 0.50ab^{A}$	$5.55 \pm 0.50ab^{AB}$	$5.27 \pm 0.72ab^{A}$	$5.33 \pm 0.66ab^{A}$	$5.08 \pm 0.62b^{AB}$
14	$5.98 \pm 0.60a^{A}$	$5.95 \pm 0.64a^{A}$	$5.90 \pm 0.73a^{A}$	$5.68 \pm 0.67a^{A}$	$5.41 \pm 0.54a^{A}$	$5.45 \pm 0.49a^{A}$
21	$4.78 \pm 0.19a^{B}$	$4.15 \pm 0.21a^{B}$	$4.65 \pm 1.48a^{BC}$	$4.10 \pm 0.85a^{B}$	$4.15 \pm 1.20a^{B}$	$4.13 \pm 0.23a^{BC}$

Dose effect on Mold & Yeast population during storage at 5 C for 3 weeks

Storage time, day	Control	UV-C treated, kJ/m^2				
		0.6	1.2	2.4	4.8	6.0
0	$3.96 \pm 0.17a^{A}$	$3.79 \pm 0.50a^{A}$	$3.37 \pm 0.70a^{A}$	$3.30 \pm 0.99a^{A}$	$2.00 \pm 0.52b^{A}$	$1.95 \pm 0.23b^{A}$
7	$3.22 \pm 0.31a^{AB}$	$3.02 \pm 0.03ab^{B}$	$2.95 \pm 0.49ab^{A}$	$2.48 \pm 0.33ab^{B}$	$2.30 \pm 0.52b^{A}$	$1.55 \pm 0.20c^{A}$
14	$3.07 \pm 0.32a^{B}$	$2.30 \pm 0.18b^{B}$	ND	ND	ND	ND
21	$3.71 \pm 0.20a^{AB}$	$2.74 \pm 0.44b^{B}$	ND	ND	ND	ND

Mukhopadhyay et al., Food Control 44:110-117, 2014

Single and Integrated Intervention

- **Many processing technologies have been proposed but none been implemented by industry because either lack of effectiveness or adverse quality effect**
- **The treatment criteria, i.e., concentration/dose/time, requires to achieve 5 log reductions by any single treatment as recommended by FDA (NACMCF, 1999) is too intense that often result in adverse effects on quality**
- **Integrated or combination of treatments can disrupt one or more of the homeostasis mechanisms in microorganisms at the cellular level and thus can limit pathogen survival at a much lower intensities of individual treatments**
- **Integrated methods works effectively when the individual interventions aim at blocking microbial growth through different mechanism**

Integrated Intervention

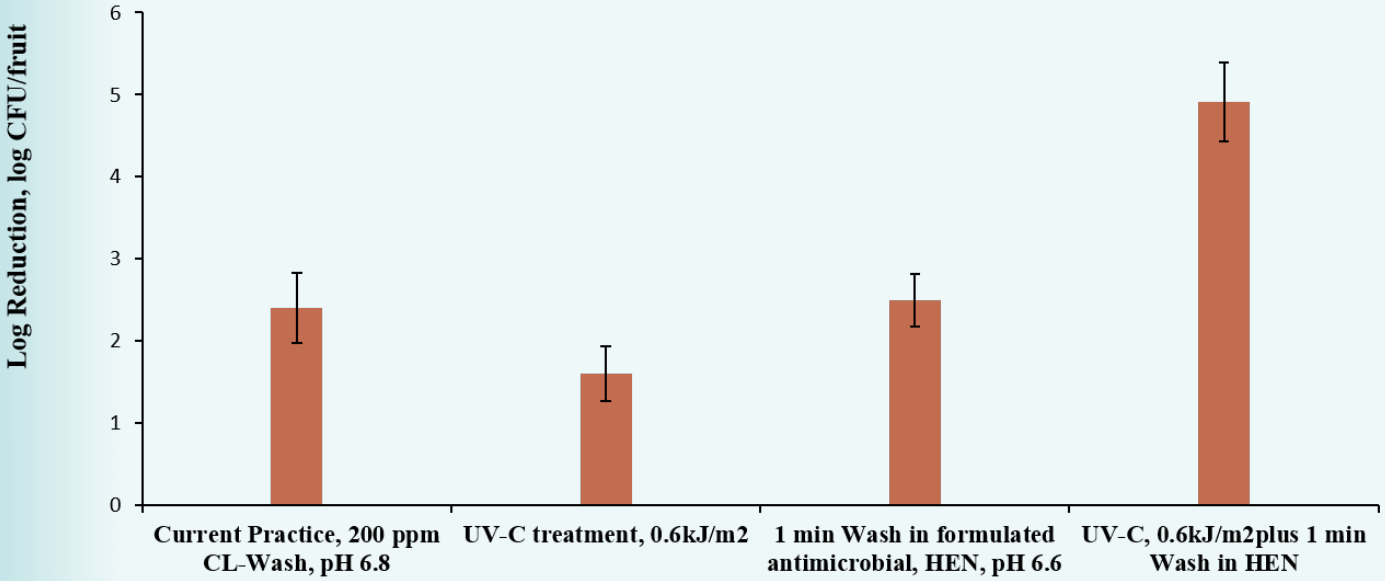
- **Integrated treatment of non thermal UV light combined with novel antimicrobial wash**
- **Intervention processing followed by antimicrobial coating or packaging**
- **Pulsed light inactivation combined with antimicrobials wash**
- **UV / PL combined with aqueous H_2O_2 or O_3 treatment for synergistic effects in an advanced oxidation process (AOP)**
- **High pressure processing with cold storage or natural antimicrobials**
- **Liquid CO_2 with active antimicrobials: CO_2 as a conveying and dispersing agent to simultaneously chill and broadcast anti-microbial**

Integrated treatment of UV-C with novel antimicrobial formulation

- **Plum tomato inoculated with a composite of three serotypes of *S. enterica* (*S.* Montevideo G4639, *S.* Newport H1275, and *S.* Stanley H0558)**
- **Treated for ~30 sec in a UV Chamber, with source emitting 90% lights at 254 nm range) and a final dose of 0.6 kJ/m^2**
- **Wash for 1 min under mild agitation in a various antimicrobial formulations**
- **Analyzed Salmonella, Background Micro flora (TAB, LAB, M&Y) and sensory quality**

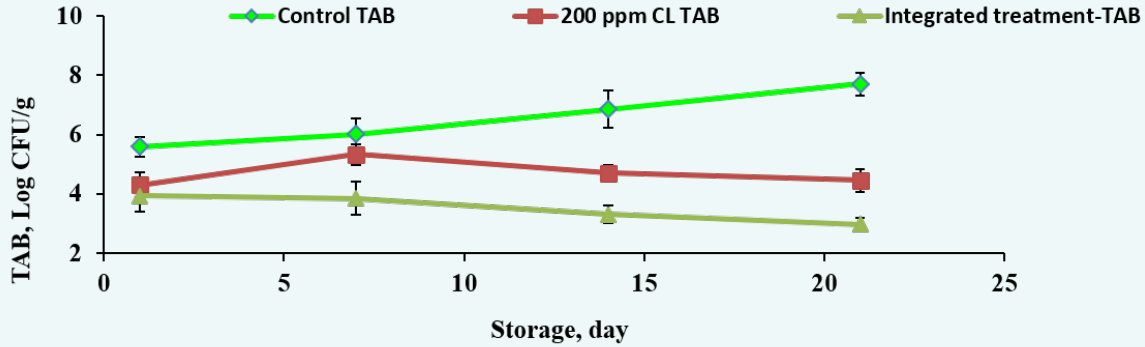
Effects of integrated treatment of UV-C light and novel antimicrobial inactivation

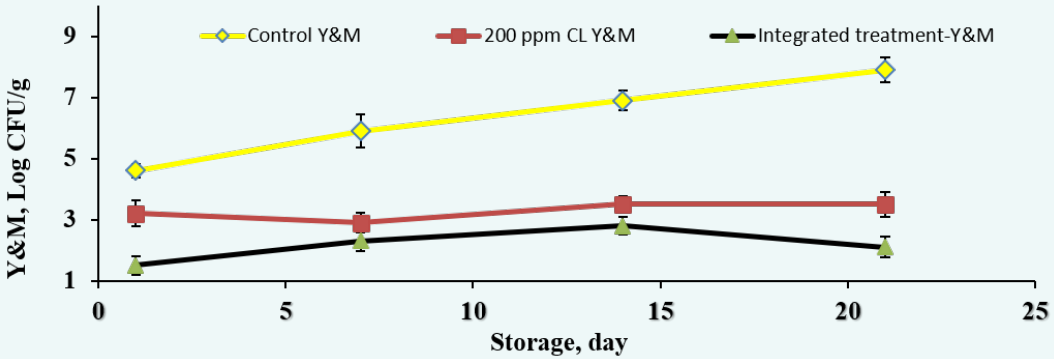
Combined treatment of 0.6kJ/m^2 UV + Wash in HEN (H_2O_2, EDTA and Nisin) @22°C for 1 min providing synergistic inactivation of *Salmonella enterica* in tomato



Mukhopadhyay et al., Food Control 56:147-154, 2015

Treatment effect on shelf life



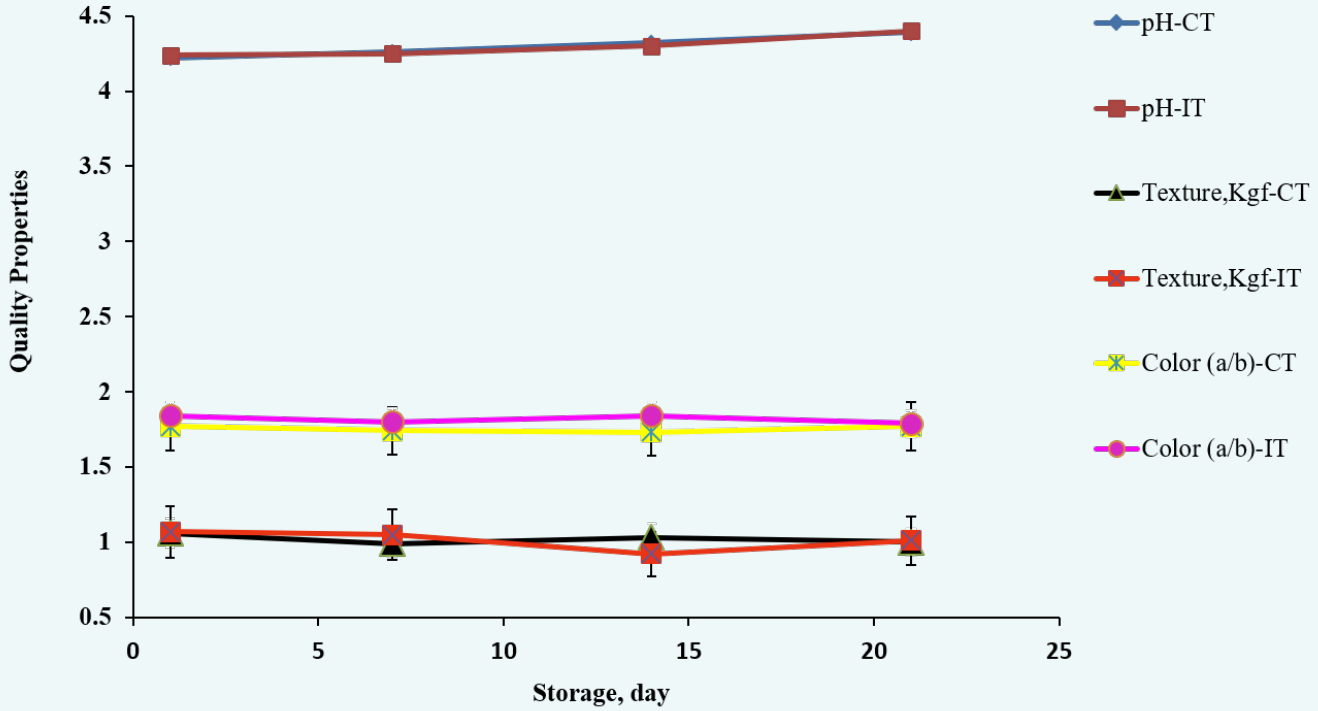


Effect of integrated treatment on total aerobic bacteria mold and yeast population during storage at room temperature (22 °C) for 21 days

Mukhopadhyay et al., Food Control 56:147-154, 2015

Quality effect

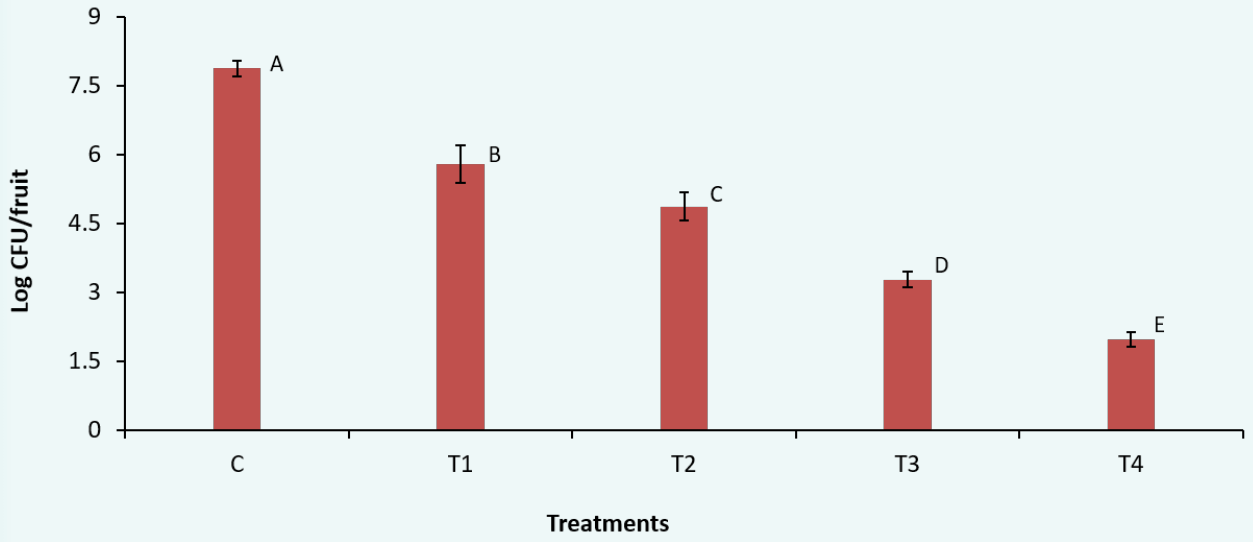
pH, Texture and Color of tomatoes after combined treatment of UV and HEN wash stored at room temperature



Mukhopadhyay et al., Food Control 56:147-154, 2015

Integrated treatment of UV light and low dose irradiation

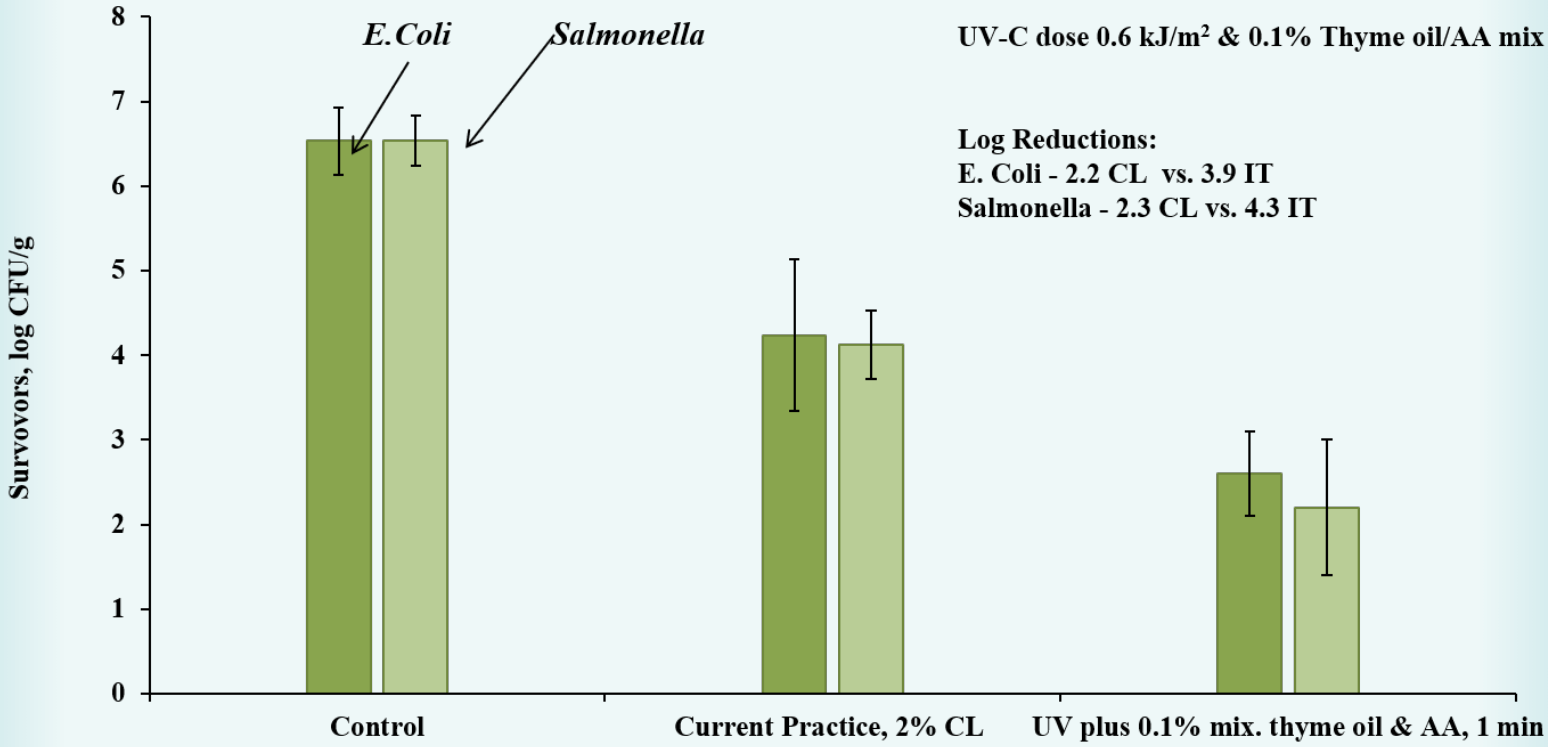
Three serotype mixture of *S. enterica* (*S.* Montevideo G4639, *S.* Newport H1275, and *S.* Stanley H0558) in tomato



UV-C dose: 0.6KJ/m^2 : Gamma irradiation doses: T 1, T2, T3,T4 are 0.1, 0.25, 0.5, 0.75 kGy

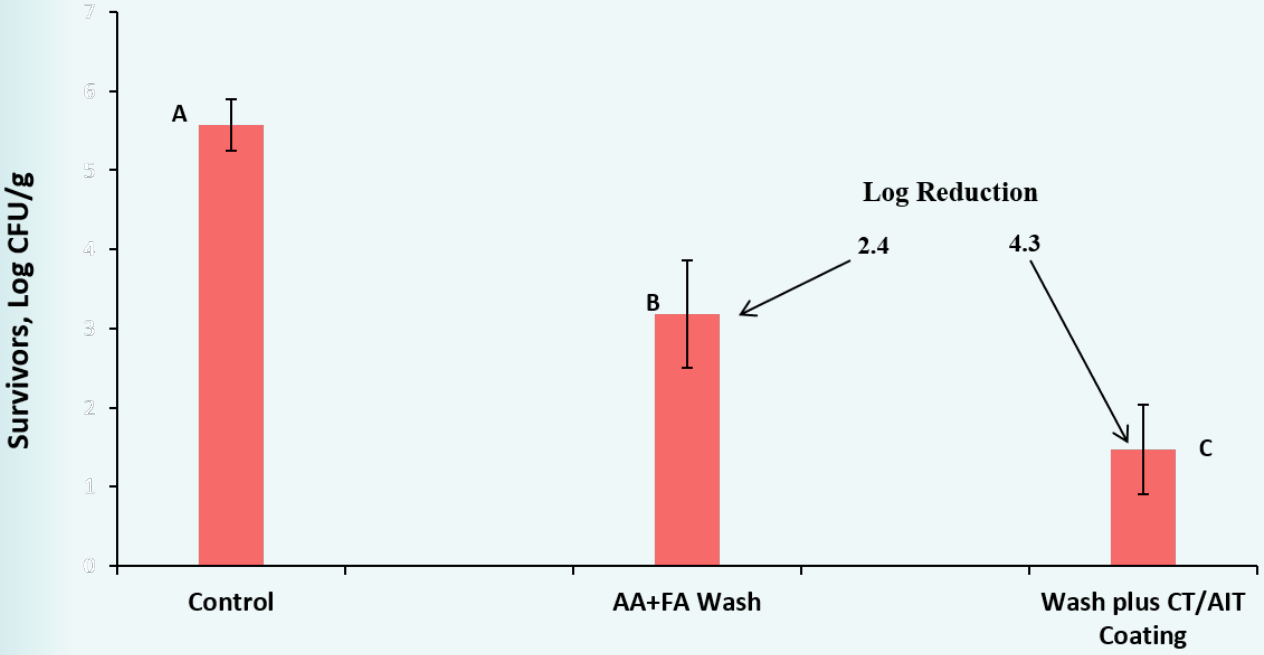
Mukhopadhyay et al., J. Food Sc. 78: M1049 -1056, 2013

Effects of integrated treatment using UV-C light & Thyme oil/AA mixture wash on inactivation of *E. coli* O157:H7 and *Salmonella* on Leafy greens



Integrated treatment effect on inactivation of a three serotype mixture of *S. enterica* (*S.* Montevideo G4639, *S.* Newport H1275, *S.* Stanley H0558) and a three strain mixture of *E. coli* O157:H7 (C9490, E02128, F00475) in Spinach leaves

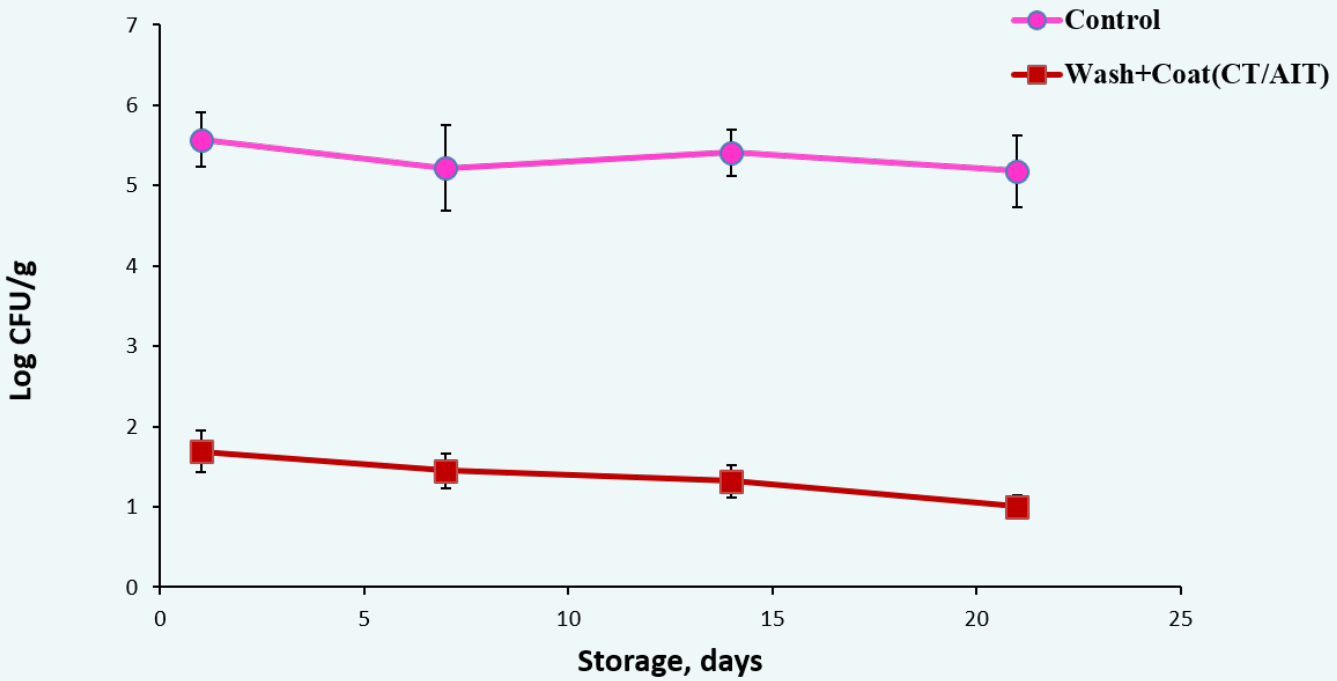
Integrated Treatment of organic acid wash and antimicrobial coating on *Salmonella* in tomato



Effects of AA+FA wash combined with Chitosan-AIT coating on inactivation of a *Salmonella enterica* cocktail on stem scars of cherry tomato

Mukhopadhyay et al., International Journal of Food Microbiology. 266:234-240, 2018.

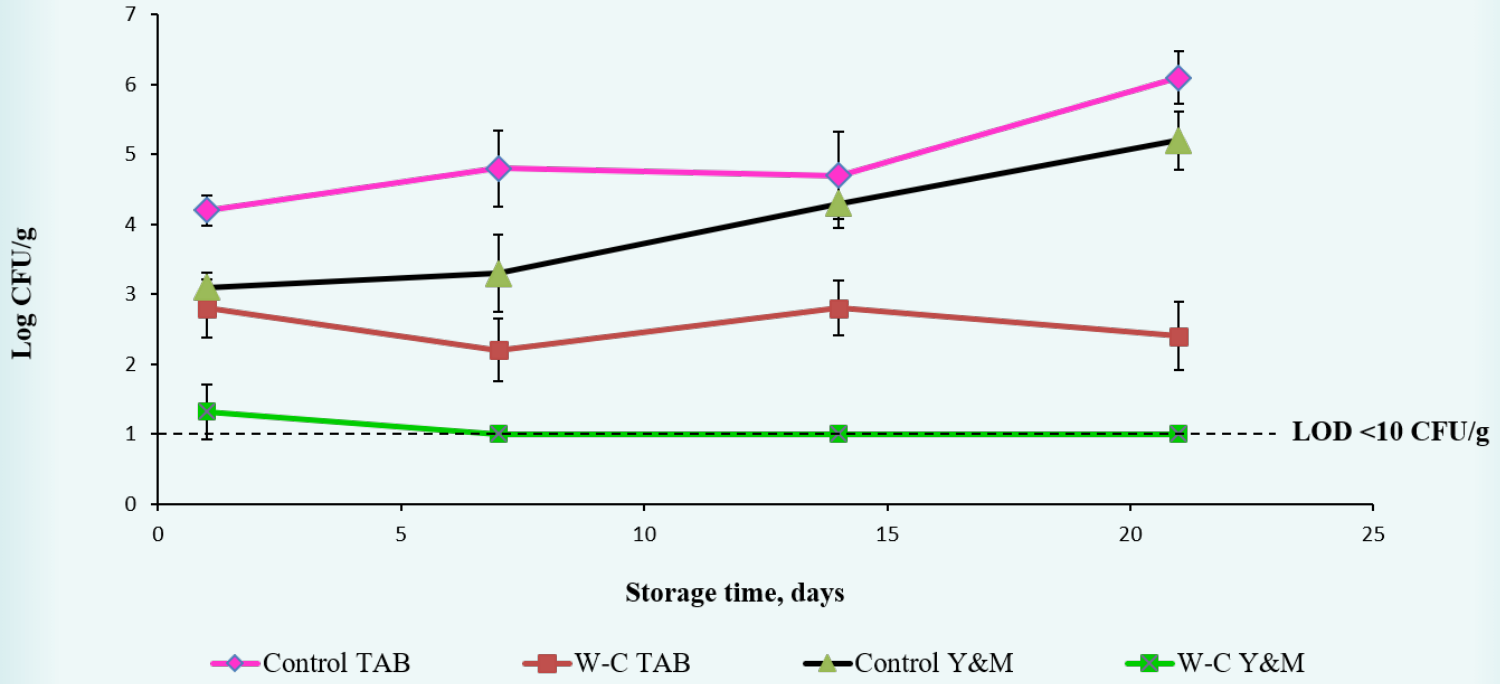
Integrated treatment effect on survival of *Salmonella* during storage



Survival of *Salmonella* composite on control and treated Tomato Stem Scars during storage at 4°C as effected by integrated treatment of organic acid and antimicrobial coating

Mukhopadhyay et al., International Journal of Food Microbiology. 266:234-240, 2018

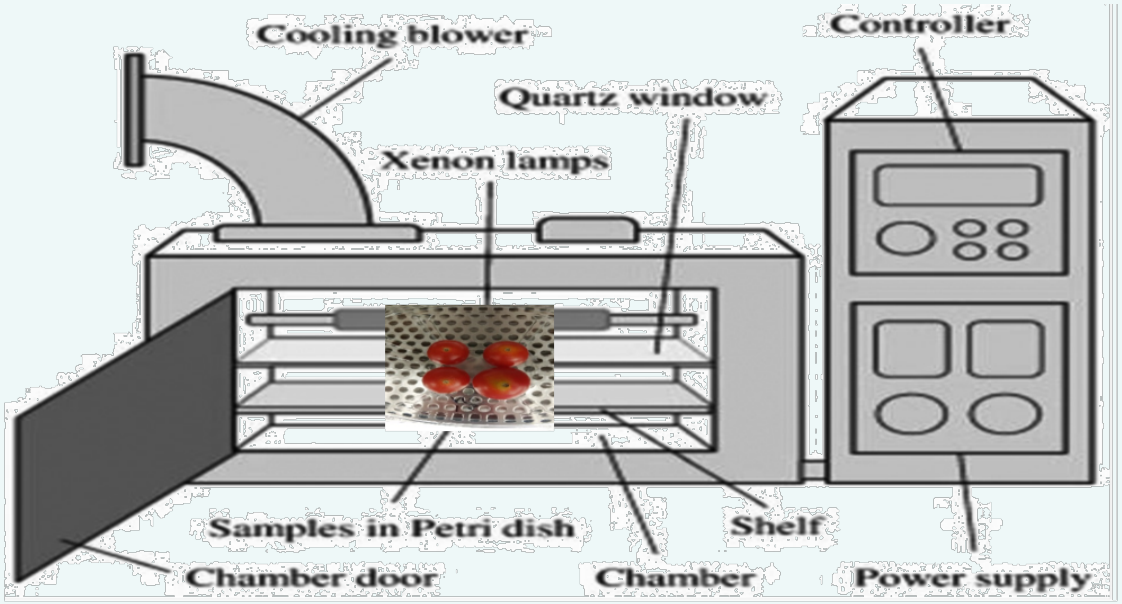
Integrated treatment effect of mix organic acid wash and Chitosan-AIT coating on background microbial load during storage

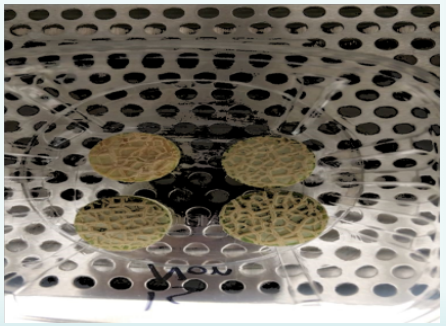


Populations of total aerobic bacteria and molds & yeasts on control and treated tomatoes during 21 day storage at 4 °C

Mukhopadhyay et al., International Journal of Food Microbiology. 266:234-240, 2018

Pulsed Light Intervention





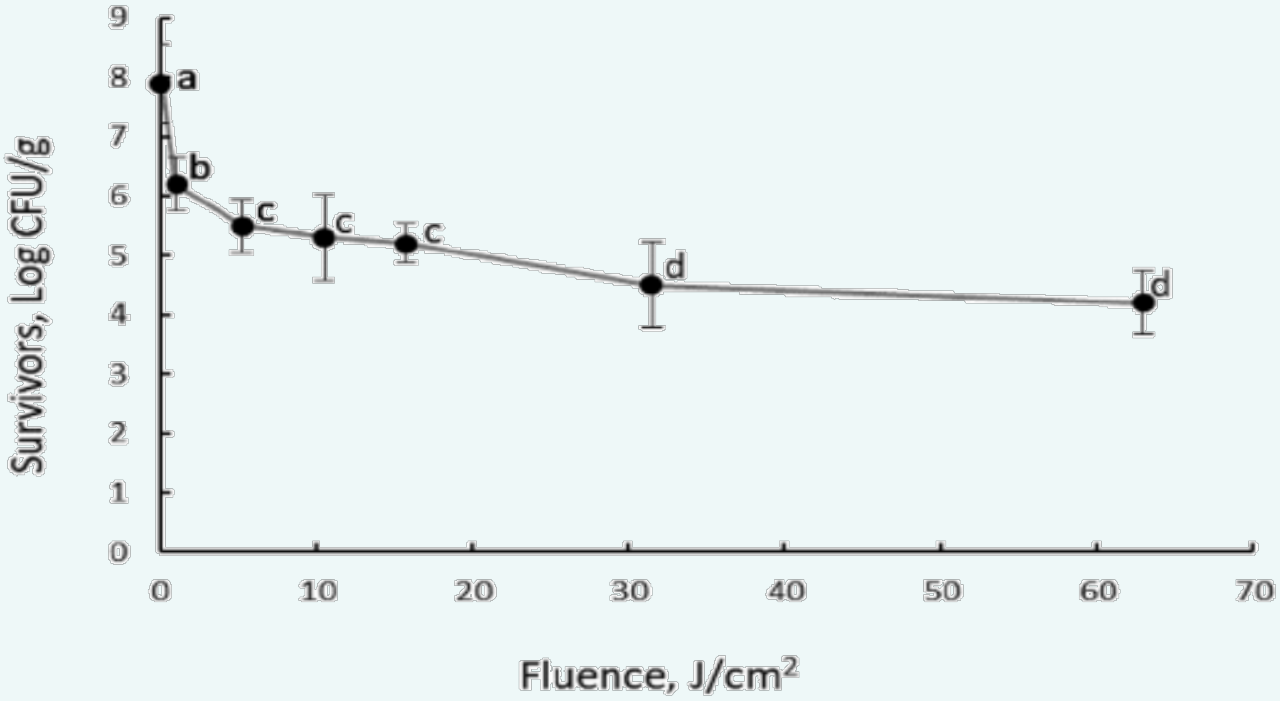
Project milestone

- Optimization of variables for pulsed light system for the inactivation of *E. coli* O157:H7*, Salmonella* spp. and/or *Listeria*, and maintenance of quality and shelf life of spinach, tomato and/or cantaloupe

- Integrated treatments combining pulsed light with aqueous antimicrobials to reduce *E. coli* O157:H7 and *Salmonella* spp. on spinach and tomato

- Integrated treatments combining aerosolized antimicrobial with pulsed light technology for inactivation of *E. coli* O157:H7 and *Salmonella* spp. on spinach and tomato

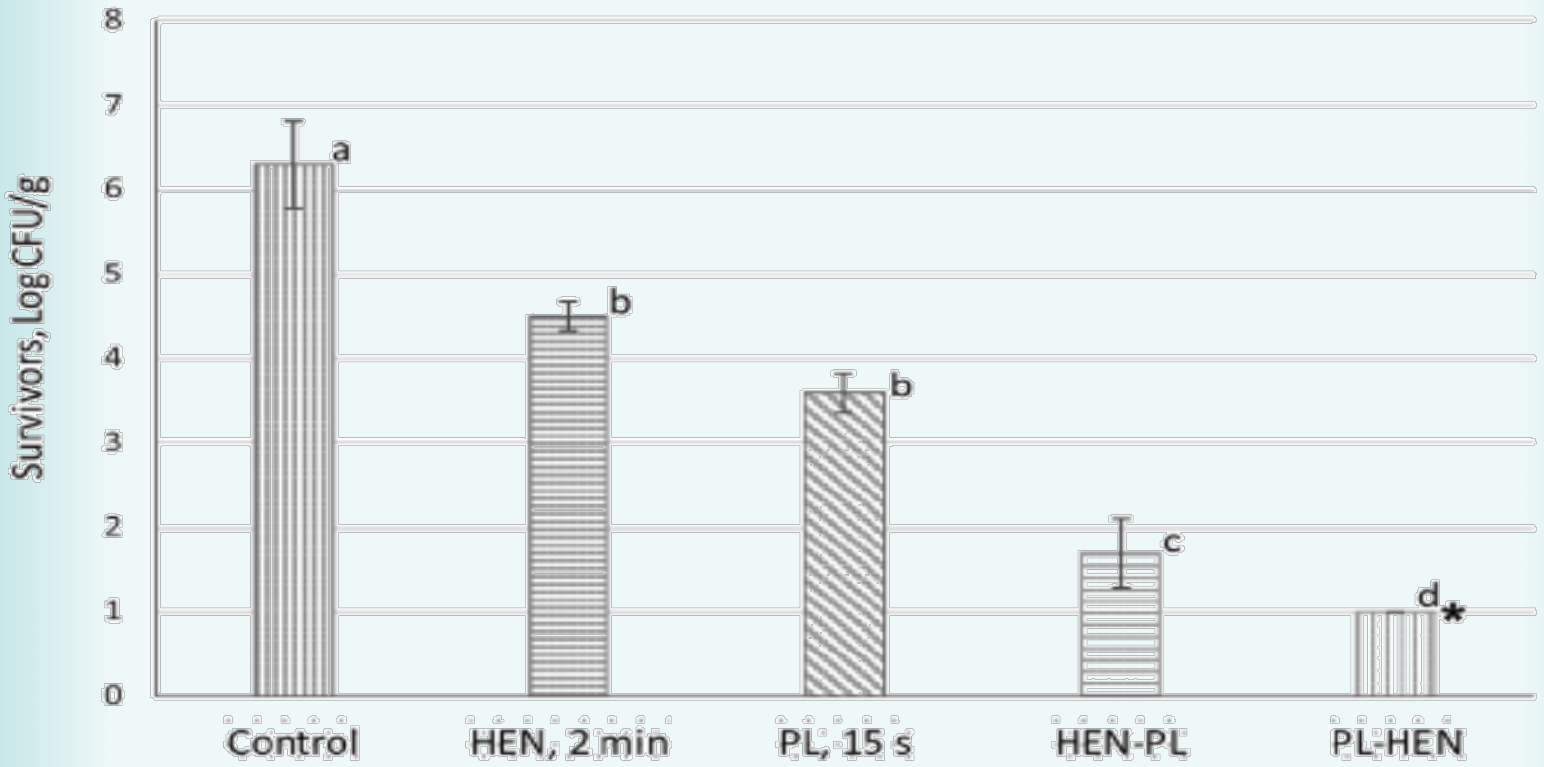
Pulsed light inactivation of pathogens in produce



Effect of PL dose treatment on *E. coli* O157:H7 on surface of spinach leaves

Mukhopadhyay et al., Food Microbiology, 82:127-134, 2019

Integrated treatment of Pulsed light and sanitizer treatment indicating safe and effective inactivation of pathogens in leafy greens



Mukhopadhyay et al., Food Microbiology, 82:127-134, 2019

Conclusion/Future Need

- ✓ Integrated treatment concepts capable delivering pasteurization level inactivation which is difficult to achieve with single technology without affecting sensory quality
- ✓ Process economy remains one of the major concern
- ✓ Develop new non-thermal integrated treatment integration for inactivation of pathogens
- ✓ Scale-up studies and validation for commercial application
- ✓ Seek industrial collaboration