Characterization, inactivation, and control of Extraintestinal Pathogenic Escherichia coli in poultry meat

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ARS Program 108 Project Name: The Role of Genotype in the Development and Validation of Growth Models and Intervention Technologies for Pathogenic Non-Shiga Toxigenic *Escherichia coli* Found in Foods.

Project Objectives:
The overall goal of this project is to determine the growth and inactivation kinetics of foodborne pathogens suspended in foods treated using thermal and nonthermal process interventions, with a strong emphasis on ExPEC.

1. Develop and validate models to simulate pathogen behavior under both growth and inactivation conditions.

2. Developing and validating non-thermal and thermal intervention technologies to inactivate pathogens and spoilage microorganisms in raw and ready-to-eat foods and food contact surfaces.

3. Examine any relationship between genotype (virulence factors) and pathogen resistance to interventions.

My Collaborators and Coauthors

- James R. Johnson, MD. Urologist, Dept. of Veterans Affairs and Univ. Minnesota.
- Dr. Lance Price, George Washington University.
- Dr. Lee-Yan Sheen & Students, National Taiwan University.
- Dr. Lihan Huang, Dr. Yanhong Liu and Jake Elder (ERRC)
- Our Staff & Students (Dr. Shiowshuh Sheen, Dr. Aixia Xu, Butch Scullen, Rommel Ramos)
- Dr. William Mackay, Edinboro University of PA

- Dr. Jack Gunther: Campy inactivation with irradiation and HPP
- FDA-CVN: Irradiation of pet treats

- Stakeholders: FSIS, CDC, FDA, Women's Health Groups, Public Health Community, Food Producers

Escherichia coli Types

- Commensal (harmless background microflora)

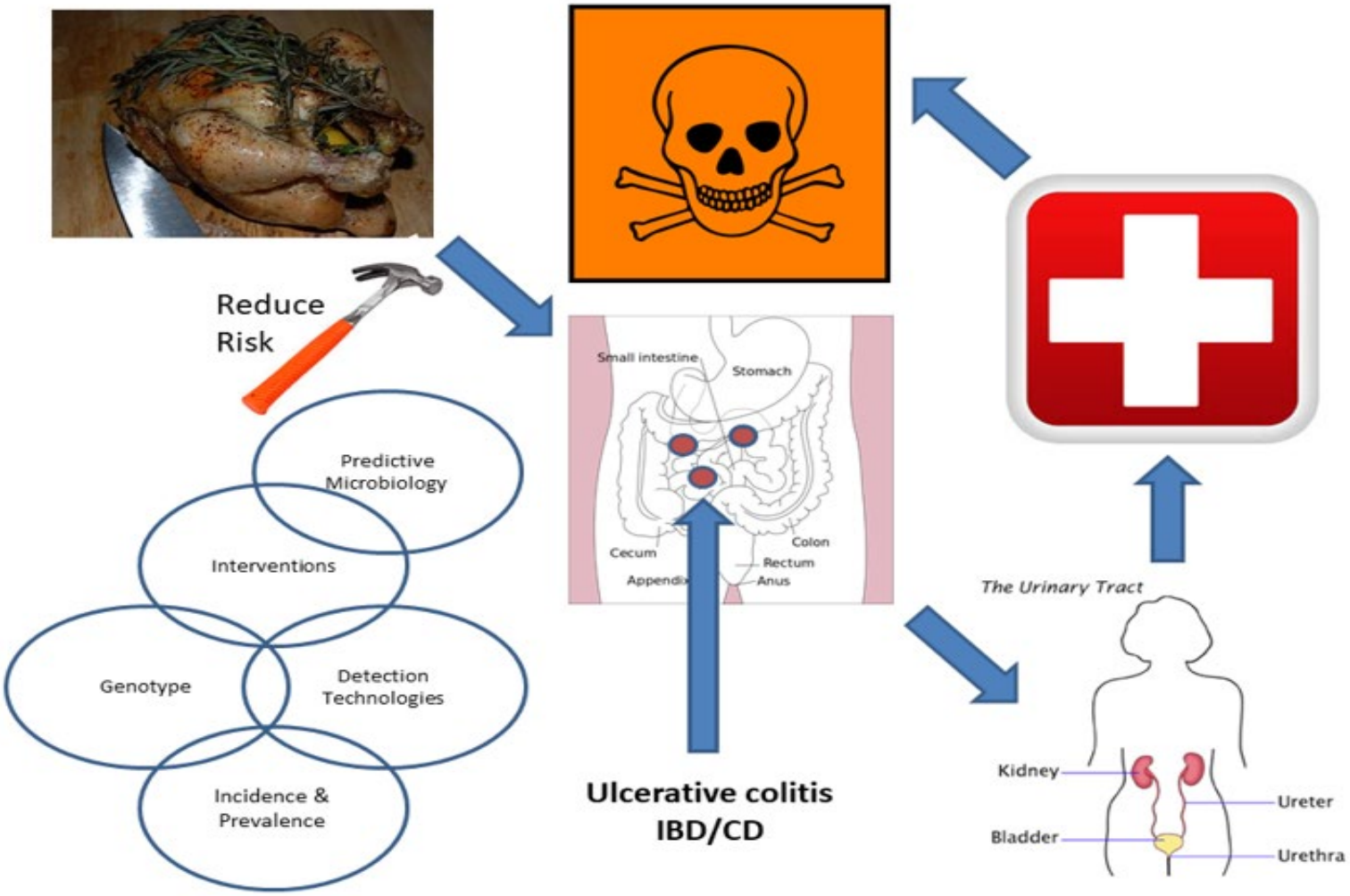
- Intestinal Pathogenic *E. coli* (iPEC)
  - STEC (B_1)
  - EHEC
  - VTEC

- Extraintestinal Pathogenic *E. coli* (B_2 *and D*)
  - Uropathogenic *E.coli (UPEC)*
  - Neonatal meningococcal *E. coli* (NMEC)
  - Avian pathogenic *E. coli* (APEC)
  - Sepsis-associated (SEPEC)

- Different O:H groups

- Hybrids (Carry both iPEC and ExPEC Virulence Factors)

Path to Illness (ExPEC)



Estimated Number of Illnesses and Deaths

	Illnesses	Hospitalizations	Deaths
Diarrheal *E. coli (STEC, etc)*	ca. 306,000	ca. 3700	ca. 31
Uropathogenic	> 10 million	ca. 100,000	ca. 23,000
Meningococcal	-	-	ca.75

Scallan et al. (2011)
Nordstom et al. (2013)

Sepsis is the 11th leading cause of death in the US

Commonalities between iPEC and ExPEC

	iPEC	ExPEC
Meat and Poultry	x	x
Produce	x	x
Seafood	x	x
Soil	x	x
Groundwater	x	x
Foodborne	x	x
Animal to Animal	x	x
Animal to Human	x	x
Human to Human	x	x

Incidence and Prevalence of ExPEC in Foods

Escherichia coli and ExPEC in 1648 Retail Food Samples (Johnson et al., 2005).

Food Type	No. of samples containing E. coli.	Samples containing of antibiotic resistant E. coli.	No. of samples containing ExPEC.	No. of samples containing E. coli with UTI O-antigens.
Miscellaneous (N=1315)	N=121 (9.2%)	N=31 (2.4%)	N=5 (0.38%)	N=12 (0.91%)
Beef/Pork (N=138)	N=95 (68.8%)	N=73 (52.9%)	N=18 (13.0%)	N=13 (9.4%)
Poultry (N=195)	N=180 (92.3%)	N= 165 (84.6%)	N=83 (42.6%)	N=28 (14.3%)

Produce ca. 1% ExPEC with UTI O-antigen and VF.

ExPEC and *K. pnuemoniae* and Inflammatory Bowel

IBD is responsible for ca. 1.3 million medical office visits, 92, 000 hospitalizations, with direct and indirect costs of 6.3 and 5.5 billion, respectively (CDC, 2010).

Mirsepasi-Lauridsen et al 2016. Extraintestinal pathogenic *Escherichia coli* are associated with intestinal inflammation in patients with ulcerative colitis. Scientific Reports, 6, 31152; doi: 10.1038/srep31152.

T. Rashid, A. Ebringer, H. Tiwana, and M. Fielder, "Role of Klebsiella and collagens in Crohn's disease: a new prospect in the use of low-starch diet," European Journal of Gastroenterology and Hepatology, vol. 21, no. 8, pp. 843-849, 2009.

de Silva et al 2017. Uropathogenic Escherichia coli pathogenicity islands and other ExPEC virulence genes may contribute to the genome variability of enteroinvasive *E. coli*. BMC Microbiology (2017) 17:68. doi: 10.1186/s12866-017-0979-5

Danese et al 2005. Extraintestinal manifestations in inflammatory bowel disease. World J Gastroenterol 11(46):7227-7236

Foodborne vs. UTI

- Liu, C. M., Stegger, M., Aziz, M., Johnson, T. J., Waits, K., Nordstrom, L., … Price, L. B. (2018). *Escherichia coli ST131-H22* as a foodborne uropathogen. mBio, 9(4), e00470-18. https://doi.org/10.1128/MBIO.00470-18

- Davis, G., Waits, K., Nordstrom, L., Weaver, B., Aziz, M., Gauld, M., Grande,H., Bigler,,R., Horwinski, J., Porter, S., Stegger, M., Johnson, J., Liu, C., Price, L. 2015. Intermingled *Klebsiella pneumoniae* populations between retail meats and human urinary tract infections. Clin. Infect. Dis. p. 1 - 8. DOI: 10.1093/cid/civ428.

- Poulsen et al. (2012), *Enterococcus faecalis* clones in poultry and in humans with urinary tract infections, Vietnam. Emerging Infectious Diseases • www.cdc.gov/eid • Vol. 18, No. 7, July 2012.

- Hedman, P., Ringertz, O., Olsson, K., Wollin, R., 1991. Plasmid-identified *Staphylococcus saprophyticus* isolated from the rectum of patients with urinary tract infections. Scand. J. Infect. Dis. 23, 569–72.

Urinary Tract Infections

- ca. >10 million cases in the US annually
- >130-175 million cases world wide annually
- Ca. 80% caused by *E. coli*
- *ca. 5% each caused by Staphylococcus saprophyticus or*
- *Klebsiella pneumoniae, E. faecalis*
- Primarily affect women and girls (75%)
- Account for 1-2 percent of medical office visits (sporadic)
- 50 % of women will have a UTI in their lifetime
- 25% will have a recurrent infection

Urinary Tract Infections (2)

- Chance of UTI increases with onset of puberty (women) do to sexual activity
- Self infection process due to transfer of feces from the anus to the
- vagina and urethra (4-5 cm distance)
- Isolates from UTI, bladder, kidney infections are typically genetic match
- the *E .coli, S. saprophyticus, K. pneumoniae* in the individual's fecal microflora
- Increased chance of UTI due to catheterization (men and women)
- Underlying health conditions
- Conclusion: Its all about contaminated feces going where its shouldn't go.
- Question: How do these bacteria get into the GI tract?

Foodborne Isolates Cause UTI and other Diseases in Animal Model Systems

- Davis, G., Waits, K., Nordstrom, L., Weaver, B., Aziz, M., Gauld, M., Grande,H., Bigler,,R., Horwinski, J., Porter, S., Stegger, M., Johnson, J., Liu, C., Price, L. 2015. Intermingled *Klebsiella pneumoniae* populations between retail meats and human urinary tract infections. Clin. Infect. Dis. p. 1 - 8. DOI: 10.1093/cid/civ428.

- Stromberg, Z. R., Johnson, J. R., Fairbrother, J. M., Kilbourne, J., Van Goor, A., Curtiss, R., & Mellata, M. (2017). Evaluation of *Escherichia coli* isolates from healthy chickens to determine their potential risk to poultry and human health. PLOS ONE, 12(7), e0180599. https://doi.org/10.1371/journal.pone.0180599

- Vincent, C., Boerlin, P., Daignault, D., Dozois, C. M., Dutil, L., Galanakis, C., … Manges, A. R. (2010). Food reservoir for Escherichia coli causing urinary tract infections. Emerging Infectious Diseases, 16(1), 88–95. https://doi.org/10.3201/eid1601.091118

- Jakobsen, L., Spangholm, D. J., Pedersen, K., Jensen, L. B., Emborg, H.-D., Agersø, Y., … Frimodt-Møller, N. (2010b). Broiler chickens, broiler chicken meat, pigs and pork as sources of ExPEC related virulence genes and resistance in Escherichia coli isolates from community-dwelling humans and UTI patients☆. International Journal of Food Microbiology, 142(1–2), 264–272. https://doi.org/10.1016/j.ijfoodmicro.2010.06.025

Adhesins	
Adhesion siderophore	iha
Dr binding adhesins	afa/draBC
E. coli common pilus	ecpA
F1C fimbriae	foc gene cluster
Heat-resistant haemagglutinin	hra
M fimbriae	bmaE
N-acetyl d-glucosamine-specific fimbriae	gaf
P fimbriae	papACEFG
S fimbriae	sfa/sfaS
Temperature sensitive haemagglutinin	tsh
Type 1 fimbriae	fimH

Dale & Woodward, 2015. Extra-intestinal pathogenic Escherichia coli (ExPEC): Disease, carriage and clones. J. Infection. Volume 71, Issue 6, Pages 615–626. .doi.org/10.1016/j.jinf.2015.09.009

Iron acquisition systems

Aerobactin receptor	iutA
Peri-plasmic iron binding protein	sitA
Salmochelin receptor	iroN
Siderophore receptor	ireA
Yersiniabactin receptor	fyuA

Dale & Woodward, 2015. Extra-intestinal pathogenic Escherichia coli (ExPEC): Disease, carriage and clones. J. Infection. Volume 71, Issue 6, Pages 615–626. .doi.org/10.1016/j.jinf.2015.09.009

Protectins and invasins

Colicin V	cva
Conjugal transfer surface exclusion protein	traT
Group 3 capsule	kpsMT II
Increased serum survival	iss
Invasion of brain endothelium	ibeA
K1/K2/K5 group 2 capsule variants	K1/K2/K5 genes
kpsM II group 2 capsule	kpsM II
Outer membrane protease T	ompT

Dale & Woodward, 2015. Extra-intestinal pathogenic Escherichia coli (ExPEC): Disease, carriage and clones. J. Infection. Volume 71, Issue 6, Pages 615–626. .doi.org/10.1016/j.jinf.2015.09.009

Toxins

α-haemolysin	hylD
Cytolethal distending toxin	cdtB
Cytotoxic necrotising factor	cnf1
Enteroaggregative E. coli toxin	astA
Haemolysin A	hylA
Secreted autotransporter toxin	sat
Serine protease	pic
Vacuolating toxin	vat

Dale & Woodward, 2015. Extra-intestinal pathogenic Escherichia coli (ExPEC): Disease, carriage and clones. J. Infection. Volume 71, Issue 6, Pages 615–626. .doi.org/10.1016/j.jinf.2015.09.009

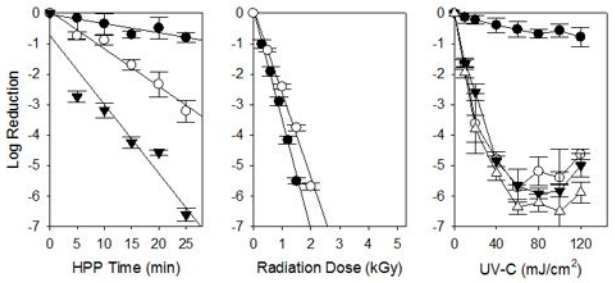
Others

β-glucoronidase	uidA
Colibactin synthesis	clb & clbB
Uropathogenic-specific protein	usp
Flagellin variant	H7 fliC
Maltose and glucose-specific PTS transporter subunit IICB	malX
Pathogenicity island marker	malX
d-serine deaminase	DsdA

Dale & Woodward, 2015. Extra-intestinal pathogenic Escherichia coli (ExPEC): Disease, carriage and clones. J. Infection. Volume 71, Issue 6, Pages 615–626. .doi.org/10.1016/j.jinf.2015.09.009

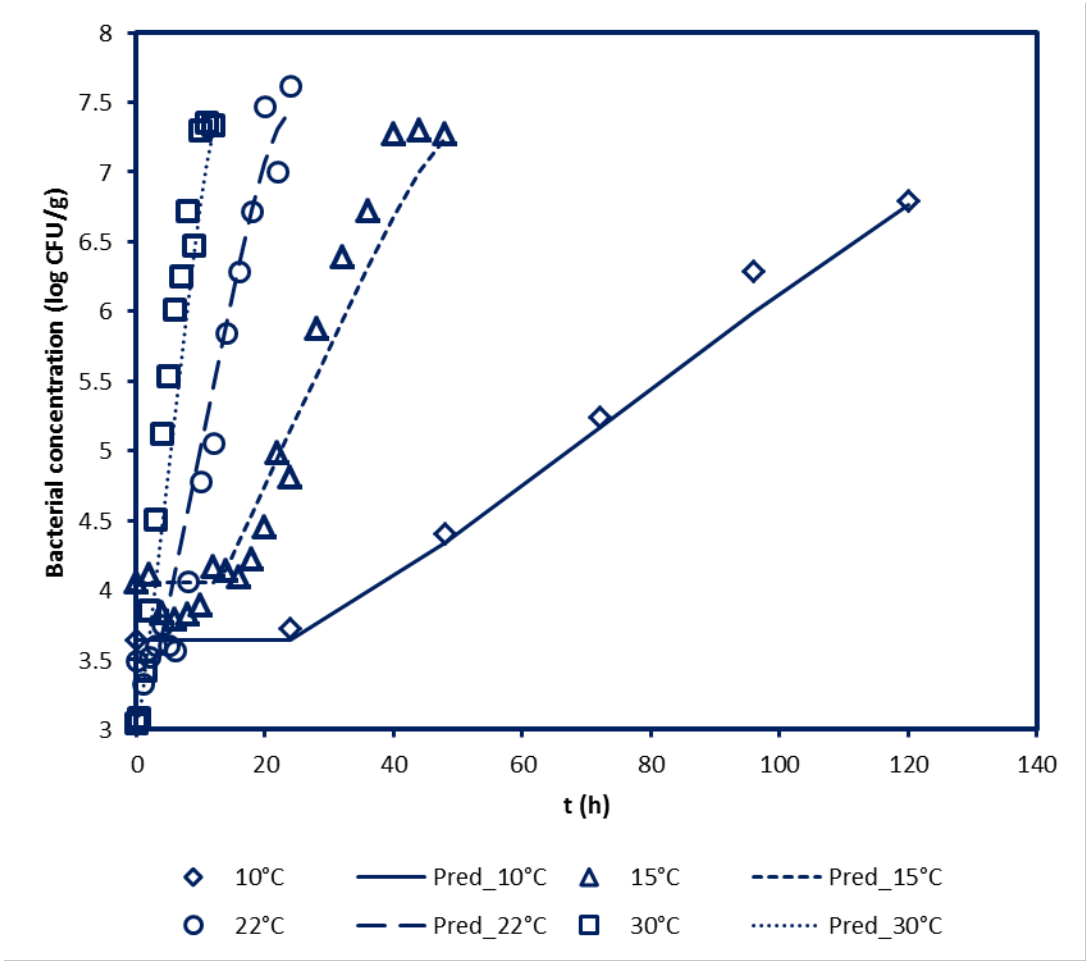
Inactivation of UPEC (Nonthermal)

Technology	Parameter	D_{10} (SEM)
High Pressure Processing	300 MPa	30.6 (±0.12) min
	400 MPa	8.37 (±1.06) min
	500 MPa	4.4 (±0.1.2) min
Gamma Radiation	4 °C	0.28 (±0.01) kGy
	-20 °C	0.36 (±0.01) kGy
Ultraviolet Light (Chicken Purge)	Stainless Steel	11.9 (±0.49) mJ/cm²
	HDPP	11.4 (±0.47) mJ/cm²
	HDPE	12.9 (±0.59) mJ/cm²



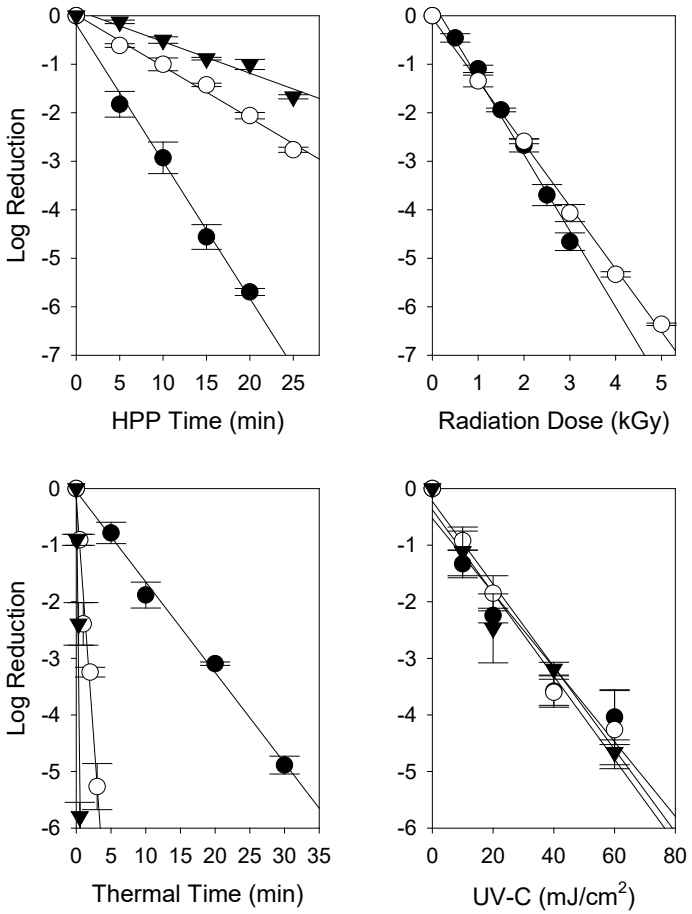
Sommers C, Scullen O, Sheen S (2016) Inactivation of Uropathogenic Escherichia coli in Ground Chicken Meat Using High Pressure Processing and Gamma Radiation, and in Purge and Chicken Meat Surfaces by Ultraviolet Light. Front. Microbiol. 7:413. doi: 10.3389/fmicb.2016.00413

UPEC Growth Curves and Model for Ground Chicken



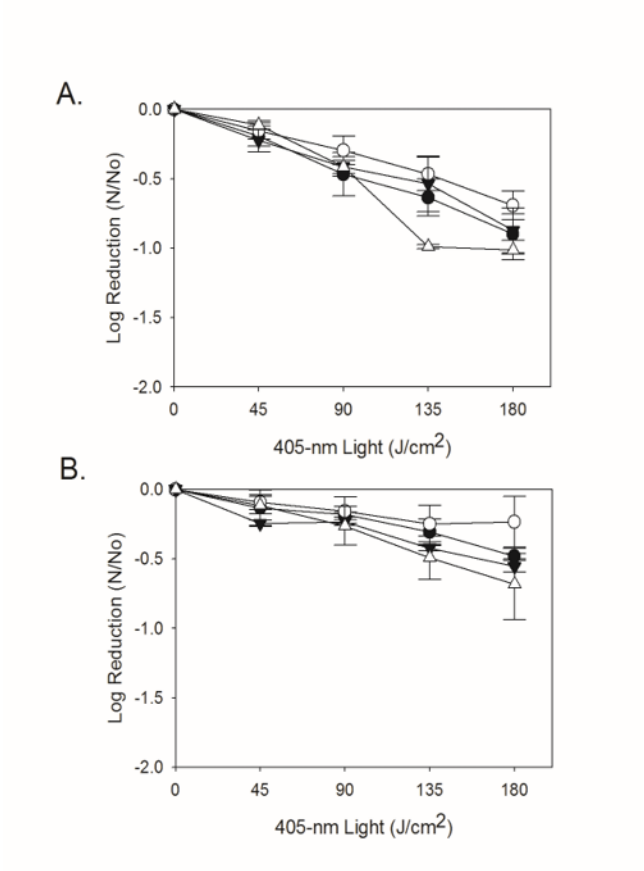
Christopher Sommers, Chi-Yun Huang, Lee-Yan Sheen, Shiowshuh Sheen, Lihan Huang. 2018. Growth modeling of Uropathogenic Escherichia coli in ground chicken Meat. Food Control 86: 397-402.

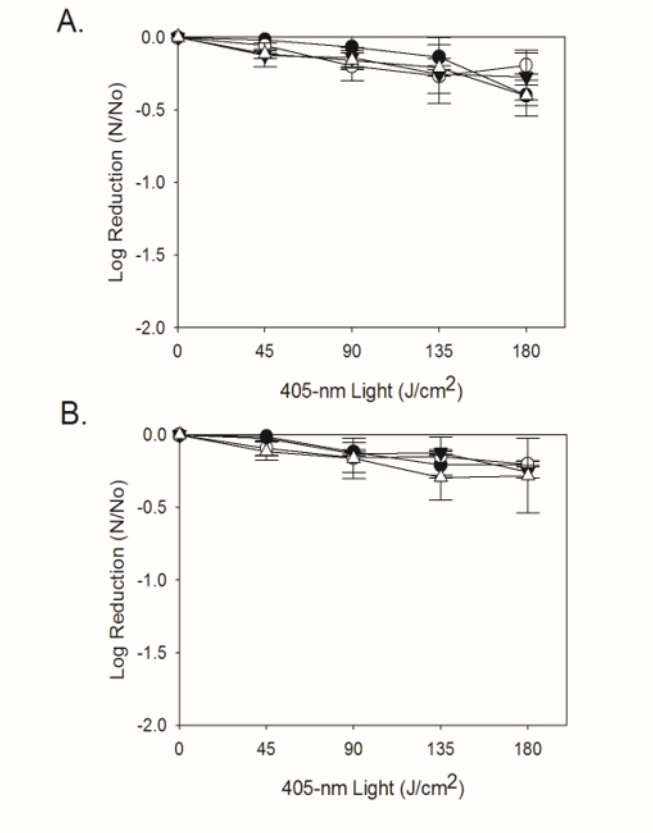
Sommers, C., Scullen, O., Sheen, S. and Mackay, W. Inactivation of *Staphylococcus saprophyticus* in chicken meat and purge using thermal processing, high pressure processing, gamma radiation, and ultraviolet light (254 nm) Food Control 75: 78 - 82. 2017.



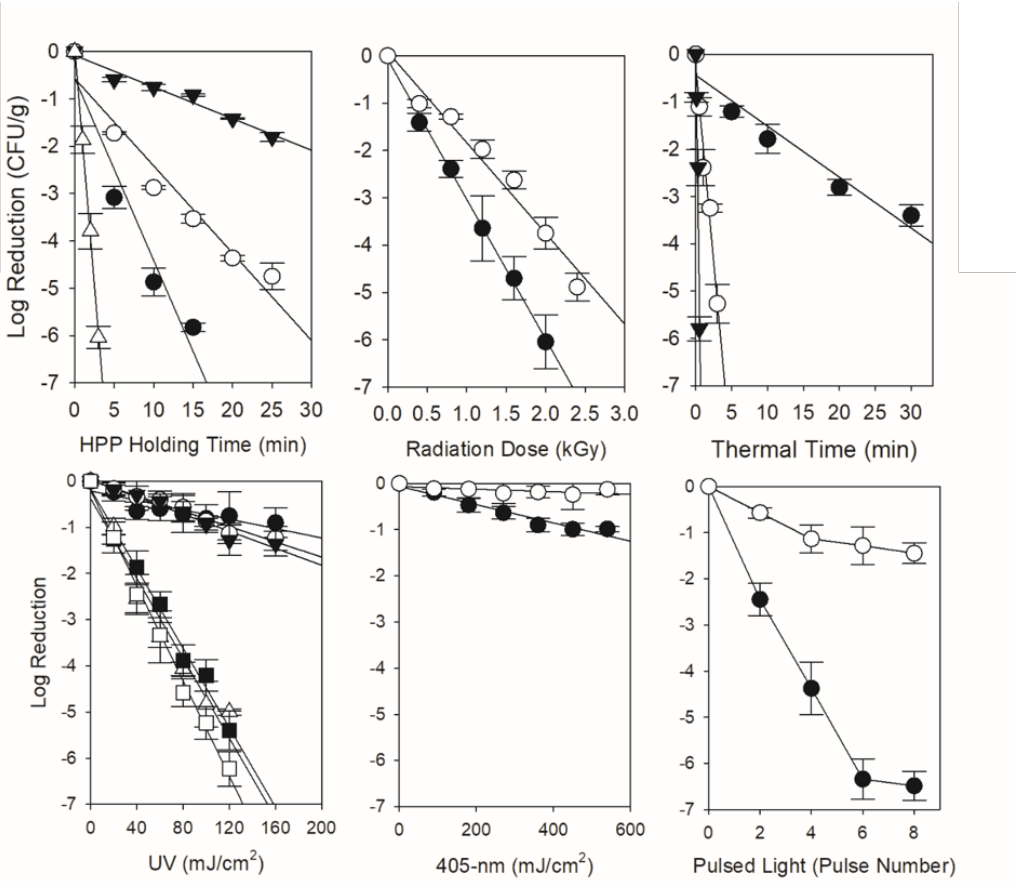
Technology	Parameter	D_{10} (SEM)
High Pressure	200 MPa, 5 °C	15.5(±0.65) min[a]
	300 MPa, 5 °C	9.43 (±0.22) min[b]
	400 MPa, 5 °C	3.54 (±0.21) min[c]
Gamma Radiation	5 °C	0.64 (±0.01) kGy[a]
	-20 °C	0.77 (±0.01) kGy[b]
Thermal Processing	50 °C	6.26 (±0.21) min[a]
	55 °C	0.60 (±0.03) min[b]
	60 °C	0.09 (±0.01) min[c]
254 nm Ultraviolet Light	SS	18.5 (±1.27) mJ/cm^{2a}
	HDPE	16.6 (± 1.54) mJ/cm^{2a}
	HDPE	14.9 (±1.88) mJ/cm^{2a}

Sommers, C., Gunther, N., Sheen, S. Inactivation of *Salmonella* spp., pathogenic *Escherichia coli*, *Staphylococcus* spp., or *Listeria monocytogenes* in chicken purge or skin using a 405-nm LED array. Food Microbiol. 64: 135

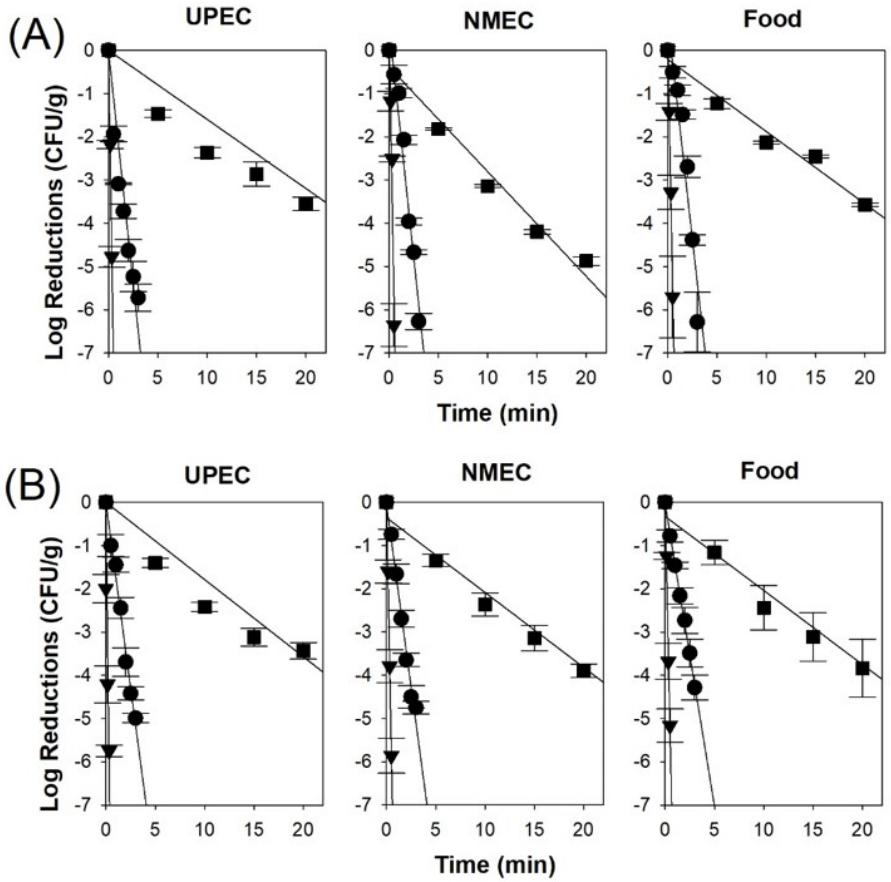




Inactivation of *K. pneumoniae* in Chicken Meat and Purge (In preparation)



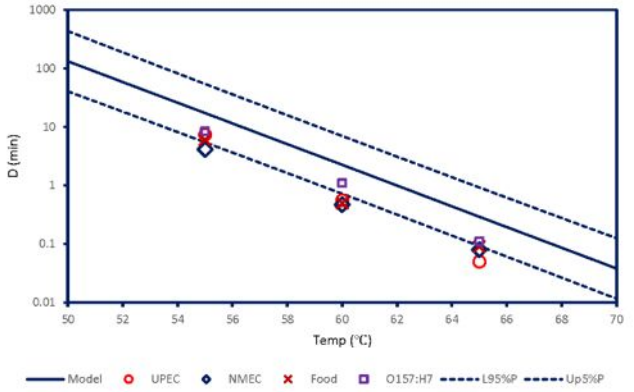
Thermal Inactivation of Multi-isolate Cocktails in Chicken Meat (Xu et al. Food Control-In Review)



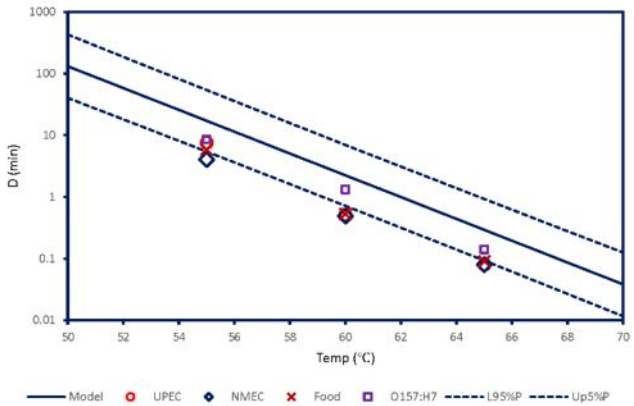
D_{10} values for Multi-isolate Cocktails

	55 °C D_{10} (min)	55 °C R^2	60 °C D_{10} (min)	60 °C R^2	65 °C D_{10} (min)	65 °C R^2	z-value (°C)
EC							
UPEC	7.34 (±0.41)	0.97	0.56 (±0.04)	0.95	0.05 (±0.01)	0.95	4.69
NMEC	4.13 (±0.08)	0.97	0.47 (±0.01)	0.96	0.08 (±0.01)	0.92	5.89
Food	5.99 (±0.12)	0.97	0.50 (±0.04)	0.91	0.09 (±0.01)	0.99	5.53
O157:H7	8.43 (±0.12)	0.96	1.10 (±0.04)	0.94	0.11 (±0.03)	0.94	5.62
APC							
UPEC	7.65 (±0.36)	0.96	0.52 (±0.02)	0.95	0.08 (±0.01)	0.95	4.62
NMEC	4.05 (±0.19)	0.95	0.49 (±0.03)	0.96	0.08 (±0.01)	0.96	5.59
Food	5.91 (±0.20)	0.97	0.53 (±0.01)	0.91	0.09 (±0.01)	0.95	5.63
O157:H7	8.62 (±0.20)	0.95	1.32 (±0.03)	0.93	0.14 (±0.02)	0.95	5.88

Fit vs. Huang Model Thermal Inactivation vs Fat Content (Huang, L., Hwang, C.-A., & Fang, T. (2019). Improved estimation of thermal resistance of *Escherichia coli* O157:H7, *Salmonella* spp., and *Listeria monocytogenes* in meat and poultry – the effect of temperature and fat and a global analysis. *Food Control*, *96*, 29–38. https://doi.org/10.1016/j.foodcont.2018.08.026



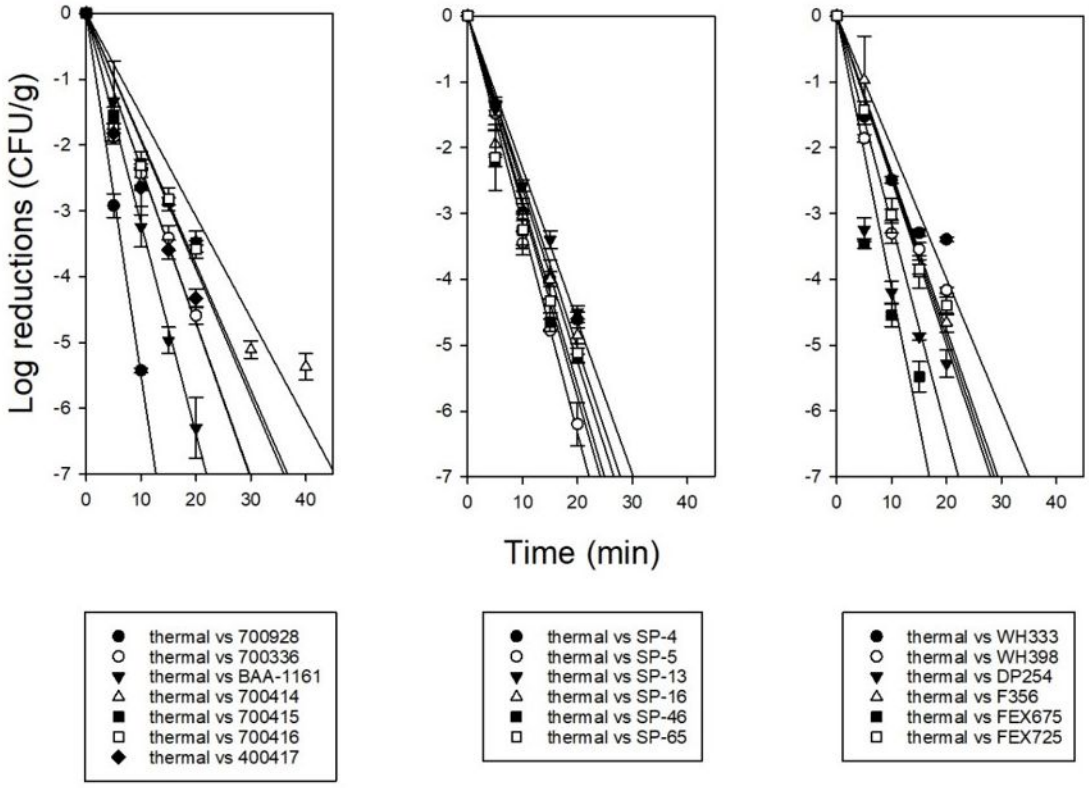
EC Petrifilms



APC Petrifilms

Figure 2

Thermal Inactivation of Individual Isolates (55 °C)



Virulence Factors and Antibiotic Resistance

Virulence Factors		Function	UPEC							NMEC							FS			
			700928	700361	BA-116 1	700414	700445	700416	700417	SP-4	SP-5	SP-13	SP-16	SP-46	SP-65	WH3313	WH3398	DP2554	F356	FEX6675	FEX7225
Adhesins	*iha*	Adhesion siderophore	Y		Y						Y			Y							
	FimH	Type 1 fimbriae	Y	Y	Y						Y			Y							Y
	papA	Fimbrial adhesin	Y	Y		Y	Y	Y	Y	Y	Y	Y	Y	Y	Y		Y	Y			
	papC	Fimbrial adhesin	Y	Y		Y	Y	Y	Y	Y	Y	Y	Y	Y	Y		Y	Y		Y	
	papG	Fimbrial adhesin	Y	Y			Y	Y	Y												
	sfa	S fimbriae	Y	Y		Y	Y	Y	Y	Y	Y	Y	Y	Y	Y				Y		
	sfaS	S fimbriae				Y	Y			Y		Y	Y		Y				Y		
	afa	Afimbrial adhesin														Y					
	PilV	Shufflon system plasmid conjugative transfer pilus tip adhesin		Y							Y		Y	Y		Y		Y	Y	Y	
	fdeC	Intimin-like adhesin				Y	Y	Y	Y	Y		Y	Y		Y	Y	Y	Y	Y		Y
Toxins	*cnf1*	Cytotoxic necrotising factor		Y		Y	Y	Y	Y												
	hlyA	Cytolysin (rtx toxin)	Y	Y		Y	Y	Y	Y												
	Vat	Vacuolating toxin	Y	Y		Y	Y	Y	Y	Y							Y	Y		Y	
	Sat	Secreted autotransporter toxin	Y		Y						Y			Y							
	senB	Plasmid-encoded enterotoxin		Y	Y						Y			Y							
Siderophores	*ireA*	Siderophore receptor ire	Y				Y	Y	Y								Y	Y		Y	
	iroN	Salmochelin receptor	Y	Y		Y	Y	Y	Y	Y		Y	Y		Y	Y	Y	Y	Y	Y	Y
	yncD	Outer membrane receptor for siderophores			Y	Y	Y	Y	Y								Y	Y	Y		Y
Colicins	*mchB*	Microcin H47 part of colicin H	Y	Y			Y	Y	Y												
	mchC	MchC protein	Y	Y			Y	Y	Y												
	mchF	ABC microcin transporter protein	Y	Y			Y	Y	Y			Y	Y		Y	Y		Y	Y	Y	
	mcmA	Microcin M part of colicin H	Y	Y		Y	Y	Y	Y								Y				
	cba	Colicin B											Y				Y		Y	Y	
	cma	Colicin M											Y				Y		Y	Y	Y
Additional virulence factors	*colicin*	Colicin V production protein		Y		Y		Y		Y		Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
	YejF	ABC microcin transporter protein	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
	gad	Glutamate decarboxylate	Y	Y	Y		Y	Y	Y	Y	Y	Y	Y	Y	Y	Y		Y	Y	Y	Y
	pic	Serine protease	Y														Y				
	chuA	Ligand gated channel protein	Y		Y	Y	Y	Y	Y												
	eilA	*Salmonella* HilA homolog		Y							Y			Y							Y
	air	Enteroaggregative immunoglobulin repeat protein		Y							Y			Y							Y
	lpfA	Long polar fimbriae		Y	Y						Y			Y			Y				Y
	iss	Increased serum survival	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
	capU	Hexosyltransferase homolog												Y							
	tsh	Temperature sensitive haemagglutinin																			Y
	T1 fimrial	Colonization of host tissue		Y		Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
	EcpD	Promotes attachment to the host cells	Y	Y	Y	Y	Y	Y	Y	Y		Y	Y		Y	Y		Y	Y	Y	Y
	iutA	Aerobactin receptor	Y		Y		Y			Y	Y	Y	Y			Y	Y	Y	Y	Y	Y
	kpsM II	Group 2 capsule synthesis	Y			Y				Y	Y	Y	Y	Y	Y	Y		Y	Y	Y	Y
	ompT	Outer membrane protease T	Y	Y	Y					Y	Y	Y	Y		Y			Y	Y		
	ibe	Invasion of brain endothelial cells								Y		Y	Y		Y	Y			Y	Y	
	traT	Conjugative plasmids			Y	Y	Y				Y	Y	Y	Y		Y	Y	Y	Y	Y	Y
	fyu	Yersiniabactin								Y		Y	Y	Y	Y						
	sinH	Intimin-like inverse autotransporter				Y	Y	Y	Y												

Antibiotic Resistance Factors		UPEC							NMEC							FS			
		700928	700361	BA-116 1	700414	700445	700416	700417	SP-4	SP-5	SP-13	SP-16	SP-46	SP-65	WH3313	WH3398	DP2554	F356	FEX6675	FEX7225
aadA1	Aminoglycoside				y								y		y		y			
aadA5	Aminoglycoside			y														y			
mph(A)	Macrolide			y																	
sul1	Sulfonamide			y	y								y		y		y				
sul2	Sulfonamide					y				y	y	y						y		y	
dfrA17	Trimethoprim			y														y			
dfrA1	Trimethoprim												y								
tet(A)	Tetracycline				y							y			y		y				
tet(B)	Tetracycline					y														y	y
blaTEM-1B	Beta-lactam			y	y					y		y			y						y
blaTEM-1C	Beta-lactam													y							
blaCTX-M-1	Cefotaxime																y				
blaCMY-2	Beta-lactam																	y			
catA1	Phenicol			y	y																
aac(3)-VIa	Aminoglycoside																y		y		
Aph(3')	Aminoglycoside					y														y	
strB	Streptomycin					y							y							y	y
strA	Streptomycin					y							y							y	y

Difference in D_{10}-Virulence Factors

Differences in D_{10} were found between isolates possessing or lacking *fdeC*, *sinH*, *cnf1*, *gad*, *ompT, iha, fimH* and *sat*. (need isogenic knock-outs, transcriptomics and proteomics)

No differences based on AR.

Most of these has been found to be regulated in response to heat

Most are involved in biofilm formation *in vivo* (AR protection)

Resistance to HPP for cocktails and individual isolates completed

Resistance to radiation for cocktails and individual isolate completed

In-House ExPEC Survey Retail Chicken (Ca. 12% of total *E.coli*)

Thermal, High Pressure and Irradiation work completed on these

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GENOME SEQUENCES

Draft Genomic Sequence of *Escherichia coli* Sequence Type 131, Isolated from Retail Chicken Skin

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ABSTRACT *Escherichia coli* sequence type 131 (ST131) is a foodborne pathogen increasingly associated with urinary tract infections. We report here the draft genomic sequence of ST131 B7S75, isolated from retail chicken skin, including information about its virulence factors and antibiotic resistance.

Urinary tract infections affect ca. 10 million people in the United States annually, with 75% of those being women (1). There is a close relationship between the consumption of retail poultry meat and urinary tract infections in humans (2, 3). Sequence type 131 (ST131) strains, which are often antibiotic resistant, have rapidly emerged to become uropathogenic *Escherichia coli* strains of clinical significance, and they are found ubiquitously in humans, food animals, and the environment (4). Toward this end, *E. coli* ST131 B7S75, recovered from retail chicken skin in our laboratory, was subjected to genomic sequencing.

B7S75 was streaked on a Trypticase soy agar (TSA) plate and incubated at 37°C for 24 h. DNA was isolated from a single colony scraped from the TSA plate. Genomic DNA was extracted using the DNeasy blood and tissue kit (Qiagen, Hilden, Germany) and quantified in a Qubit 3.0 fluorimeter (Life Technologies, Carlsbad, CA, USA). The genomic DNA library was prepared using the Nextera DNA Flex library prep kit (Illumina, San Diego, CA, USA). Libraries were analyzed for concentration, pooled, and denatured for loading onto a flow cell for cluster generation. Denatured libraries were sequenced on the Illumina MiniSeq platform. A total of 6.7×10^6 sequencing reads using 150-bp paired-end sequencing were obtained. Read quality was assessed with FastQC version 1.0.0 (Illumina BaseSpace Labs). The genome was assembled *de novo* using SPAdes (version 3.9.0), and 189 contigs (386-fold coverage) were obtained, the longest of which was 633,173 bp. Annotation was performed using the NCBI Prokaryotic Genome Annotation Pipeline (PGAP; version 4.3) (5). The genome characteristics were genome size, 5.21 Mb; GC content, 50.66%; total genes, 5,457; total coding sequences (CDS), 5,366; number of CDS coding genes, 5,130; number of RNA genes, 91; number of rRNAs, 12; number of tRNAs, 74; number of noncoding RNAs (ncRNAs), 5; and number of pseudogenes, 236. The O and H antigen of B7S75 are O25:H4 (SerotypeFinder 2.0). The multilocus sequence type was sequence type 131 (ST131). The plasmid multilocus sequence type was IncF:F16:A–B1. Virulence factors of B7S75 associated with urinary tract infections (UTIs) include the enterobactin siderophore receptor gene (*iroN*), increased serum survival gene (*iss*), glutamate decarboxylase gene (*gadA*), periplasmic chaperone EcpD gene (*ecpD*), outer membrane protease T gene (*ompT*), intimin-like inverse autotransporter gene (*sinH*), and the type 1 fimbrial protein gene (*f1P*). Antimicrobial resistance (AR) genes include those for aminoglycosides [*aac(3)-IId*] and extended beta-lactamases (bla_{TEM-1B}) (according to the Illumina Bacterial Analysis Pipeline version 1.0.4).

Genomics data are now considered an integral part of risk assessment for food

Citation Xu A, Mackay W, Scullen O, Sheen S, Ramos R, Sommers C. 2019. Draft genomic sequence of *Escherichia coli* sequence type 131 isolated from retail chicken skin. Microbiol Resour Announc 8:e01533-18. https://doi.org/10.1128/MRA.01533-18.

Editor Jason E. Stajich, University of California, Riverside

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GENOME SEQUENCES

Draft Genomic Sequences of Nine Extraintestinal Pathogenic *Escherichia coli* Isolates from Retail Chicken Skin

Aixia Xu,[a] Shannon Tilman,[a] Kristy Wisser-Parker,[a] O. Joseph Scullen,[a] Christopher H. Sommers[a]

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ABSTRACT Extraintestinal pathogenic *Escherichia coli* strains were isolated from retail chicken skin. Here, we report the draft genomic sequences for these nine *E. coli* isolates, which are currently being used in agricultural and food safety research.

Extraintestinal pathogenic *Escherichia coli* (ExPELC) isolates, often multidrug resistant, are associated with urinary tract infections, ulcerative colitis, meningitis, and sepsis, which affect over 11 million people in the United States annually (1–3). Also, ExPEC is associated with similar veterinary diseases in addition to avian colibacillosis (4). ExPEC commonly contaminates poultry meat and other foods (5–8). Isolates from food that contain the appropriate virulence factors cause disease in animal model systems (9, 10). Control of ExPEC in food and agricultural production could reduce the incidence of ExPEC-related disease in both humans and animals. Toward this end, nine ExPEC strains recovered from retail chicken skin are currently being used for agricultural and food safety research (11).

Stock cultures were streaked onto Trypticase soy agar plates and incubated for 24 h at 37°C. Genomic DNA was extracted from single colonies using the DNeasy blood and tissue kit (Qiagen, Hilden, Germany) and quantified in a Qubit 3.0 fluorimeter (Life Technologies, Carlsbad, CA, USA). The genomic DNA library was prepared using the Nextera DNA flex library prep kit (Illumina, San Diego, CA, USA). Libraries were analyzed for concentration, pooled, and denatured for loading onto a flow cell for cluster generation. Denatured libraries (1.8 pM) were sequenced on an Illumina MiniSeq platform using the 2 × 150-bp paired-end protocol with 50× coverage. Illumina reads were assembled *de novo* using SPAdes version 3.9.0. Virulence factors, antibiotic resistance genes, genome size, N_{50} values, multilocus sequence type (MLST), noncoding RNAs (ncRNAs), rRNAs, tRNAs, genes, and coding sequences (CDSs) were determined using the Illumina Bacterial Analysis Pipeline version 1.0.4 and the NCBI Prokaryotic Genome Annotation Pipeline (PGAP) version 4.3. The accession numbers and assembly metrics are listed in Table 1.

Genomics data are now considered an integral part of risk assessment for food safety and environmental microbiology (http://www.fsis.usda.gov/wps/wcm/connect/d79eaa29-c53a-451e-ba1c-36a76a6c6434/Microbial_Risk_Assessment_Guideline_2012-001.pdf?MOD=AJPERES). These genomic data will be useful for understanding ExPEC pathogenesis and helping to elucidate its role in human and veterinary diseases.

Data availability. The whole-genome shotgun projects reported here have been deposited in DDBJ/ENA/GenBank under the accession numbers and BioProject numbers listed in Table 1. The versions described in this paper are the first versions.

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Citation Xu A, Tilman S, Wisser-Parker K, Scullen OJ, Sommers C. 2018. Draft genomic sequences of nine extraintestinal pathogenic *Escherichia coli* isolates from retail chicken skin. Microbiol Resour Announc 7:e00859-18. https://doi.org/10.1128/MRA.00859-18.

Editor Julie C. Dunning Hotopp, University of Maryland School of Medicine

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What to finish before Dec 31st, 2020

- Submit *K. pneumoniae* paper (Research Done)
- Submit ExPEC Irradiation paper (Research Done)
- Submit ExPEC High Pressure Paper (Research Done)

- Submit Ultraviolet Light Paper (222-254-282 nm comparison) (Research Done)

- Help Aixia Xu find a job
- Fill SY position vacant 3.5 years (*as if that will ever happen*)
- ExPEC and fresh produce research (Liu, Elder, Niemira)

- Work with Yanghong Liu and Jake Elder on their ExPEC biofilm and acid tolerance research

- I'm not writing another project plan

Modeling the Antimicrobial and High Pressure Processing impact on the Survival of Pathogenic *Escherichia coli* in Ground Meats

USDA/ARS/FSIS Food Safety Workshop

February 20-22, 2019

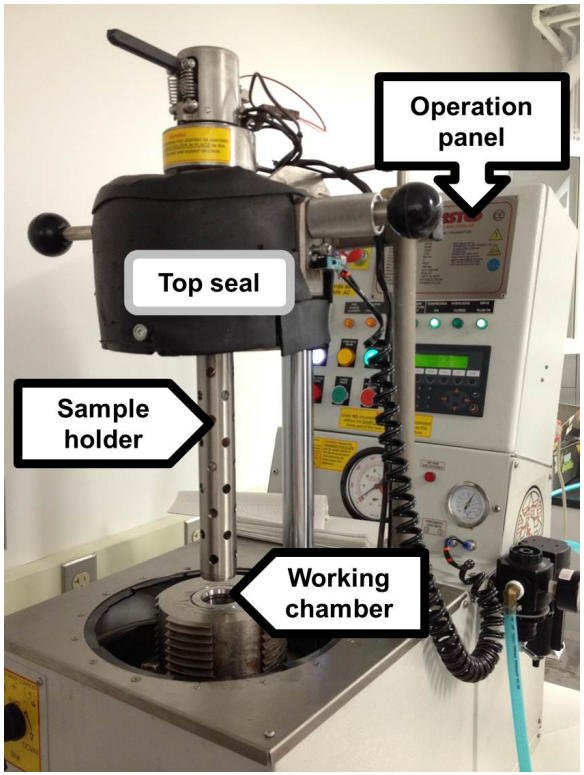
High Pressure Processing (HPP)

High Hydrostatic Pressure, Non-thermal
Unit operation to reduce foodborne
Pathogens in selected foods

Batch-type processing
Operation cost: moderate
Advantages: maintain quality and nutrients

High Pressure Processing (HPP) (Lab scale and production unit)

Mini Food lab FPG5620,
Stansted Fluid Power Ltd., Essex, UK



The AV-10 HPP System
(Production capacity @ 10MM lbs per year)
https://www.avure-hpp-foods.com/hpp-equipment/av-10/
(AVURE Technologies, Inc.)
(Example only, others available in market)

High Pressure (HPP) - products in market

(No Preservatives added)

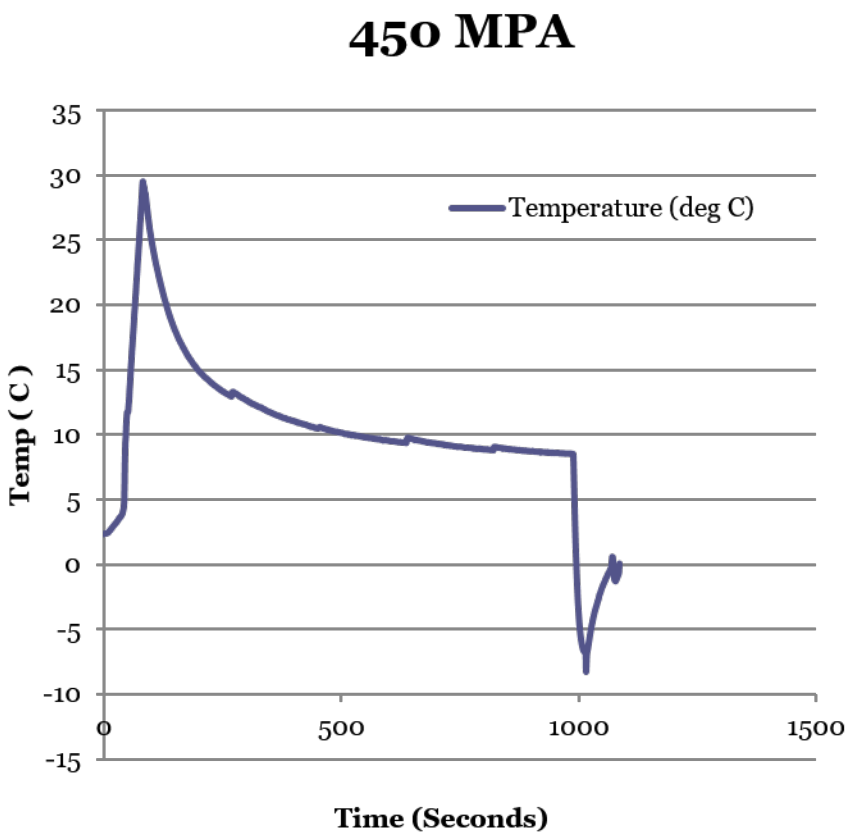
https://www.makethenaturalchoice.com/Products/Deli-Meats/Natural-Choice-Honey-Deli-Ham

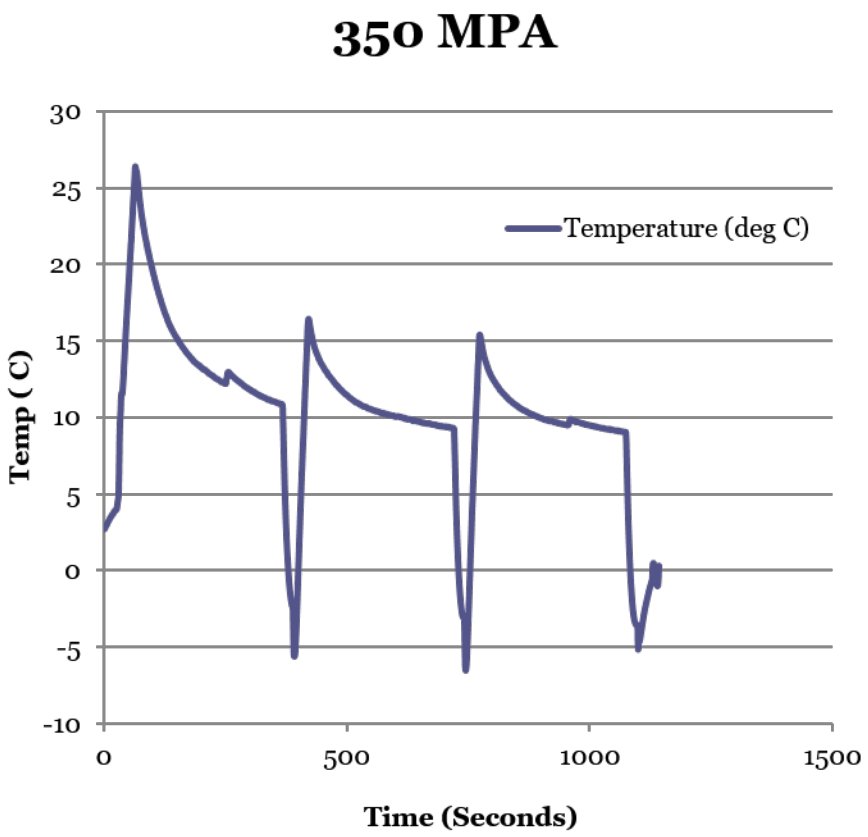
MegaMex Foods LLC, Wholly Guacamole™ products were introduced to the food service and retail markets since 1997.

>> Maintain color and texture

https://www.eatwholly.com/products/wholly-guacamole/wholly-guacamole-homestyle-guacamole/ (and etc.)

Temperature Profile during HPP Treatment (ground meat sample, Lab scale unit)





Pathogenic *Escherichia coli* strains

1. *E. coli* O157:H7 - C9490, 59762 and 59768 (strains with food outbreaks including meats);

2. UPEC - 700336, 700414 and 700415 (strains involved in Urinary Track Infection) (Sommers et al. 2016)

   (All strains obtained from the American Type Culture Collection (ATCC))

Damages of *E. coli* cells with HPP treatment (structure changes – SEM images)

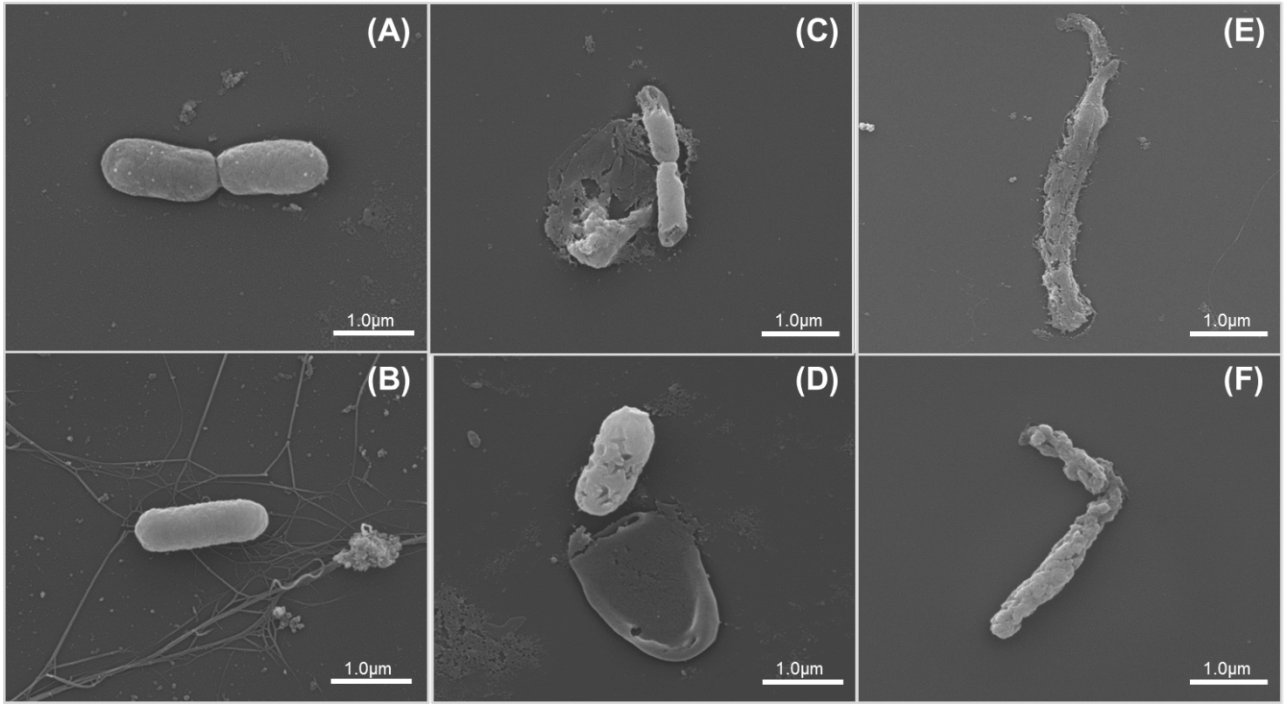


Figure A (0 MPa), C (350 MPa) and E (550 MPa): Non-O157 STEC (Big 6)
B (0 MPa), D (350 MPa) and F (550 MPa): STEC O157:H7
Process time: 15 min

Hsu H-Y, et. al., (2014). Food Microbiology, 40:25-30

Lethality due to HPP (alone)

Inactivation of the *E.coli* O157:H7 and UPEC in ground meat treated at different pressure (300-500 MPa) for 15 min

Pressure (MPa)	*E. coli* O157:H7 (in log CFU/g reduction)	UPEC (in log CFU/g reduction)
300	$0.58 \pm 0.07^{a, x}$	$0.51 \pm 0.04^{a, x}$
350	$1.64 \pm 0.04^{b, x}$	$1.62 \pm 0.09^{b, x}$
400	$2.12 \pm 0.10^{c, x}$	$2.14 \pm 0.06^{c, x}$
450	$4.26 \pm 0.06^{d, x}$	$3.35 \pm 0.06^{d, y}$
500	$7.01 \pm 0.05^{e, x}$	$5.21 \pm 0.11^{e, y}$

Chien et al. (2017). Modeling the inactivation of Escherichia coli O157:H7 and Uropathogenic E. coli in ground beef by high pressure processing and citral. Food Control 73: 672-680

Processing and Antimicrobial Hurdles

- Microbial count reduction (inactivation) can be enhanced either by applying antimicrobial agent(s), and with processing intervention to achieve "synergetic" impact.

  (assessed cell reduction via 1 + 1 > 2 or decreasing the imposed level of stresses)

  - Reduce any extreme use of a single treatment to avoid texture damages, nutrient degradations, but achieve similar or better results/goal

Damages of *E. coli* cells after HPP treatment with/without citral added (structure changes – SEM/TEM images)

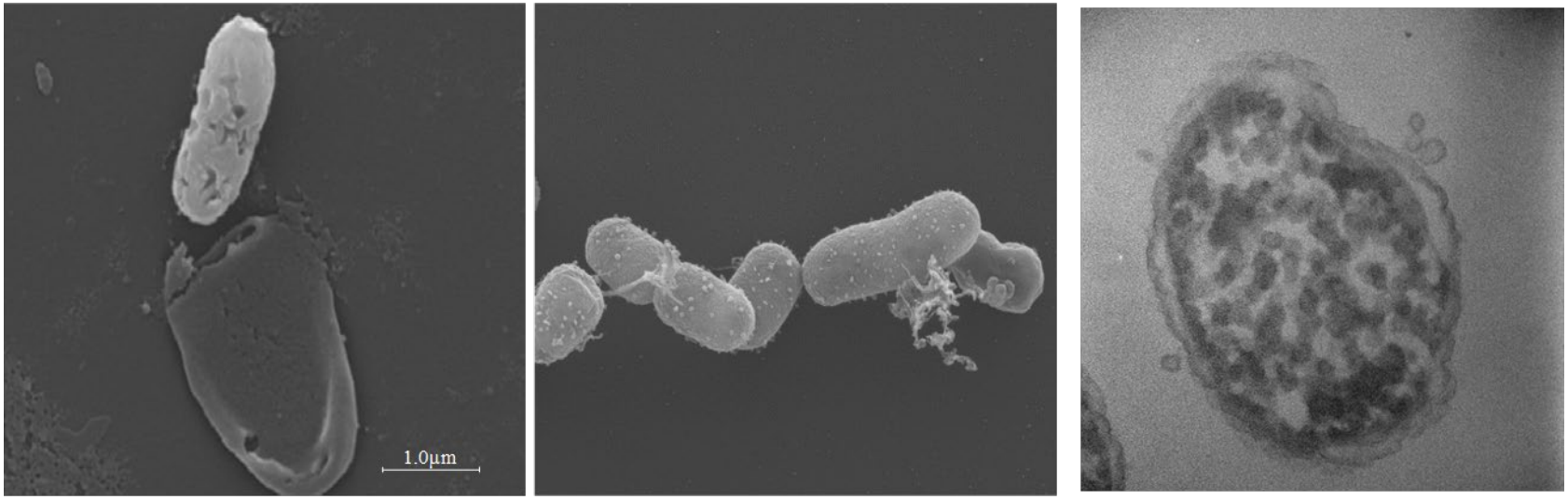


Figure: Left (350 Mpa, 15 min, w/o citral, SEM @ 15,000x); Center (350 Mpa, 15 min, w/1.0% citral, SEM @ 15,000x) Right (350 Mpa, 15 min, w/1.0% citral, TEM @ 65,000x)

Combination Effect of High-Pressure Processing and Essential Oil (Melissa officinalis extracts) or their constituents for the inactivation of E. coli in Ground Beef. Chien SY, Sheen S., Sommers C., Sheen LY. Food and Bioprocess Technology (2018).

Food-grade Additive

Antimicrobials:

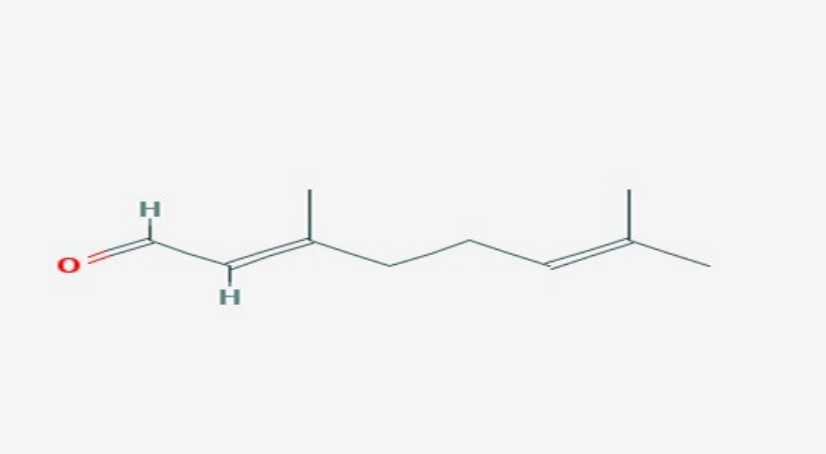
GRAS status only: applied alone or in combination

- **Natural compounds (high in demands)**

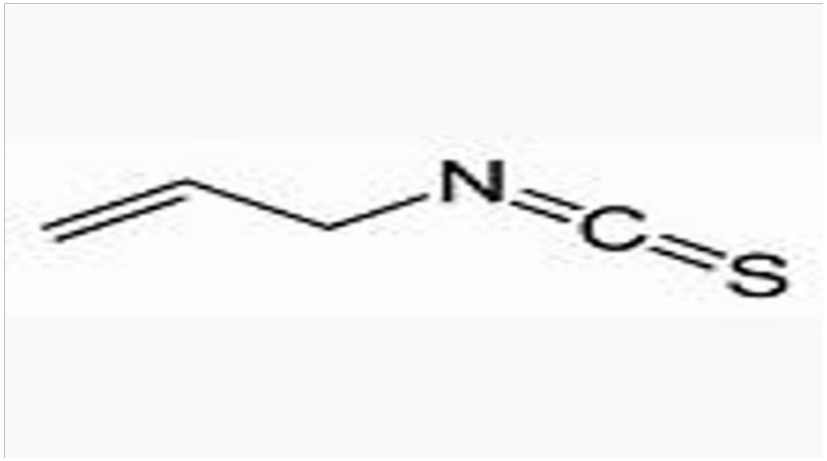
Thymol,
Citral,
Trans-Cinnamaldehyde,
Carvacrol,
Allyl Isothiocyanate,
Geraniol
etc.

Level of Impact depending on pathogen strains and processing means

Antimicrobials (examples)



Citral: $C_{10}H_{16}O$
Molar mass: 152.24 g/mol
Appearance: Pale yellow liquid
Odor: Lemon like
Density: 0.893 g/cm^3
Boiling point: 229 °C (444 °F; 502 K)



Allyl isothiocyanate: C_4H_5NS
Density: 1.01 g/cm^3

Allyl isothiocyanate is the organosulfur compound with the formula CH_2CHCH_2NCS. This colorless oil is responsible for the pungent taste of mustard, radish, horseradish, and wasabi

HPP and Antimicrobials

HPP – potentially higher operation cost and food quality damage issues; (when used alone)

Antimicrobials: natural food grade (GRAS) compounds or chemicals (weak or little impact alone)

- **Thymol** and **HPP:** inactivated *E. coli* (O157:H7 and UPEC) on fresh ground meats (beef) Frontiers in Microbiology, 2016
- **Citral** and **HPP:** Food Control, 2017
- **Trans-cinnamaldehyde** and **HPP**: J. Food Science, 2018
- **Allyl Isothiocyanate** and HPP: Frontiers in Microbiology, 2018
- **Multiple (2+: e.g. Citral** and **Geraniol):** Food and Bioprocess Technology, 2018

Model Development and Applications

Theoretical and Empirical models:

Theoretical models: difficult to develop

Based on biological, chemical, and physical theories

- Solve the governing equation with other conditions typically involve numerical solutions

Empirical models: relatively easy to develop; limited applications

Based on experimental design with key parameters

- Regression analyses to achieve linear and/or nonlinear model development or construction

Variables and Levels used in CCD (Central Composition Design)

Factor	Level -α (-1.682)	Level -1	Level 0	Level +1	Level +α (1.682)
Pressure (MPa)	215.9	250	300	350	384.1
Citral dose (% w/w)	0.58	0.75	1.00	1.25	1.42
Time (minutes)	6.59	10	15	20	23.41

Reductions (log CFU/g) of *E. coli* O157:H7 and UPEC on ground beef after HPP+Citral with Central Composite Design. (14 design combinations + 6 center points, no. 15-20)

Trail No.	Pressure MPa (level)	Conc. % (level)	Time minute (level)	Inactivation of O157:H7 (log CFU/g reduction) Log (N_o/N)	Inactivation of UPEC (log CFU/g reduction) Log (N_o/N)
1	250 (-1)	0.75 (-1)	10 (-1)	0.86±0.08	2.01±0.36*
2	250 (-1)	0.75 (-1)	20 (+1)	1.64±0.03	3.85±0.45*
3	250 (-1)	1.25 (+1)	10 (-1)	1.07±0.04	2.66±0.45*
4	250 (-1)	1.25 (+1)	20 (+1)	1.94±0.06	4.65±0.15*
5	350 (+1)	0.75 (-1)	10 (-1)	2.75±0.42	5.30±0.43*
6	350 (+1)	0.75 (-1)	20 (+1)	4.33±0.42	7.92±0.06*
7	350 (+1)	1.25 (+1)	10 (-1)	3.88±0.49	7.92±0.06*
8	350 (+1)	1.25 (+1)	20 (+1)	7.96±0.02	7.92±0.06*
9	215.9 (-α)	1.00 (0)	15 (0)	0.85±0.07	2.26±0.36*
10	384.1(+α)	1.00 (0)	15 (0)	5.47±0.55	7.92±0.06*
11	300 (0)	0.58 (-α)	15 (0)	1.74±0.15	3.82±0.39*
12	300 (0)	1.42 (+α)	15 (0)	2.66±0.15	7.92±0.06*
13	300 (0)	1.00 (0)	6.6 (-α)	1.18±0.05	2.81±0.21*
14	300 (0)	1.00 (0)	23.4 (+α)	3.60±0.51	7.92±0.06*
15	300 (0)	1.00 (0)	15 (0)	2.53±0.47	4.69±0.06*
16	300 (0)	1.00 (0)	15 (0)	2.40±0.40	4.65±0.06*
17	300 (0)	1.00 (0)	15 (0)	2.25±0.24	5.32±0.06*
18	300 (0)	1.00 (0)	15 (0)	1.97±0.02	5.52±0.06*
19	300 (0)	1.00 (0)	15 (0)	2.55±0.49	5.52±0.06*
20	300 (0)	1.00 (0)	15 (0)	2.48±0.48	5.14±0.06*

General Linear Regression Models

Experimental design: Factorial design (full or fractional)
Central composite design

Model Type: linear polynomial equation (e.g. 2 parameters)

$$Y = a X_1 + b X_2 + c X_1 X_2 + d X_1^2 + e X_2^2$$

a, b, c, d, e: constants to be determined by regression

Models for Microbial Survival or inactivation

- General Expression - Dimensionless Non-linear model
  - Lethality (Sheen's model):

$$L = k \prod_{i=1}^{n} \left[\frac{X_i - X_{\min/or\ max}}{X_i + X_{\min/or\ max}} \right]^{mi}$$

Escherichia coli O157:H7 inactivation on ground beef impacted by citral dose (C, %), Pressure (P, MPa) and process time (T, min)

Linear model (Based on CCD):

$$\text{Log}\,(N_o/N) = 27.3978 - 0.1290 \cdot P - 14.5910 \cdot C - 0.6920 \cdot T + 0.0424 \cdot P \cdot C + 0.0020 \cdot P \cdot T + 0.2580 \cdot C \cdot T + 0.0001 \cdot P^2$$

$R^2 = 0.92$ (Eq. I)

Dimensionless nonlinear model (Sheen model):

$$\text{Log}(N_o/N) = 536.30 \left[\frac{P-100.0}{P+100.0}\right]^{5.3447} \left[\frac{C-0.10}{C+0.10}\right]^{4.3370} \left[\frac{T-6.0}{T+6.0}\right]^{0.8555}$$

Sum of squared error/uncorrected total: 20.0324/ 610.1

F value = 412.35, Pr > F (< 0.0001) (Eq. II)

UroPathogenic E. coli (UPEC) inactivation on groundbeef impacted by citral dose (C, %), Pressure (P, Mpa) and process time (T, min)

Linear model (Based on CCD):

$$\text{Log}\,(N_o/N) = -15.4349 + 0.0463 \cdot P - 0.2065 \cdot C + 0.6508 \cdot T - 0.0006 \cdot P \cdot T - 0.2477\, C \cdot T + 3.5661 \cdot C^2$$

$R^2 = 0.93$ (Eq. III)

Dimensionless nonlinear model (Sheen model):

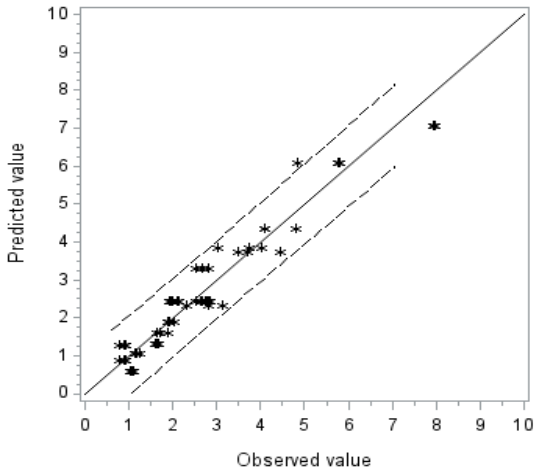
$$\text{Log}(N_o/N) = 79.5838 \left[\frac{P-100.0}{P+100.0}\right]^{2.6861} \left[\frac{C-0.10}{C+0.10}\right]^{2.4168} \left[\frac{T-6.0}{T+6.0}\right]^{0.3555}$$

Sum of squared error/uncorrected total is 37.9695/ 1918.7

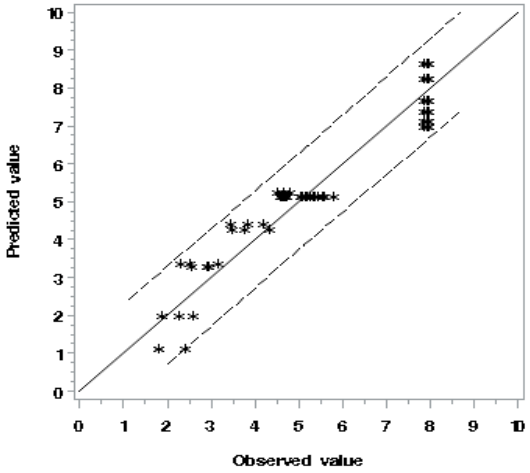
F value = 693.47, Pr > F (< 0.0001) (Eq. IV)

Model Performance: Prediction vs. Experiment data

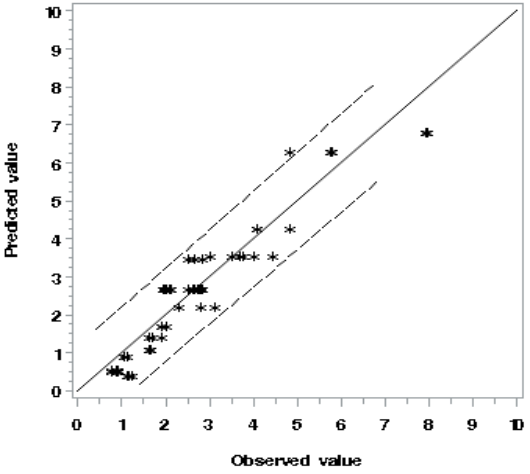
A) O157:H7 (linear model)



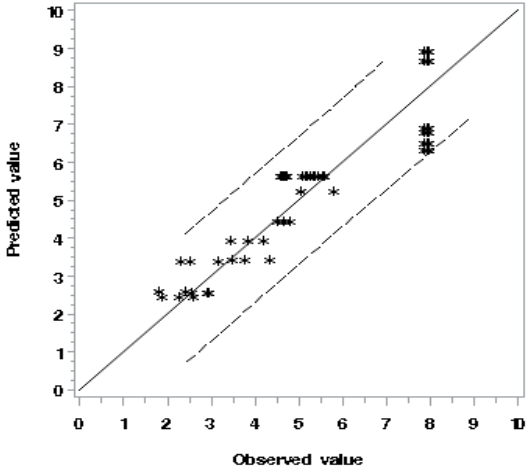
C) UPEC (linear model)



B) O157:H7 (dimensionless nonlinear model)



D) UPEC (dimensionless nonlinear model)



Experimental Validation of Predictive Models
(log reduction of *E. coli* O157:H7 and UPEC in ground beef)

Run	Variables			Log_{10} reduction (CFU/g)					
	Pressure (MPa)	Citral conc. (%)	Time (min)	E. coli O157:H7 Experiment	E. coli O157:H7 Predict (Eq. I)	E. coli O157:H7 Predict (Eq. III)	UPEC Experiment	UPEC Predict (Eq. II)	UPEC Predict (Eq. IV)
1	260	1.1	18	1.82±0.10	1.71	1.70	4.27±0.09	4.44	4.46
2	340	0.8	14	2.83±0.12	2.60	2.76	5.74±0.13	5.38	5.76
3	200	0.3	10	0.44±0.07	-	0.02	0.41±0.09	-	0.48

Variables and Levels used in CCD (Pressure, time, trans-cinnamaldehyde)

Factor	Level -α (-1.682)	Level -1	Level 0	Level +1	Level + α (1.682)
Pressure (MPa)	265.9	300	350	400	434.1
Trans-cinnamaldehyde Concentration (%)	0.1	0.2	0.35	0.5	0.6
Time (minutes)	11.5	15	20	25	28.5

Escherichia coli O157:H7 and UPEC inactivation on ground chicken impacted by Pressure (P, MPa), process time (T, min) and trans-cinnamaldehyde dose (C, %)

Linear model (Based on CCD):

$$\text{Log}\,(N_o/N) = 11.81479 - 0.10421 \cdot P + 13.95493 \cdot C - 0.03296 \cdot T + 0.00425 \cdot P \cdot C + 0.00011 \cdot P \cdot T - 0.51972 \cdot C \cdot T + 0.00019 \cdot P^2 + 6.76458 \cdot C^2 + 0.00836 \cdot T^2$$

$R^2 = 0.75$ (Eq. 1)

Dimensionless nonlinear model (Sheen's model):

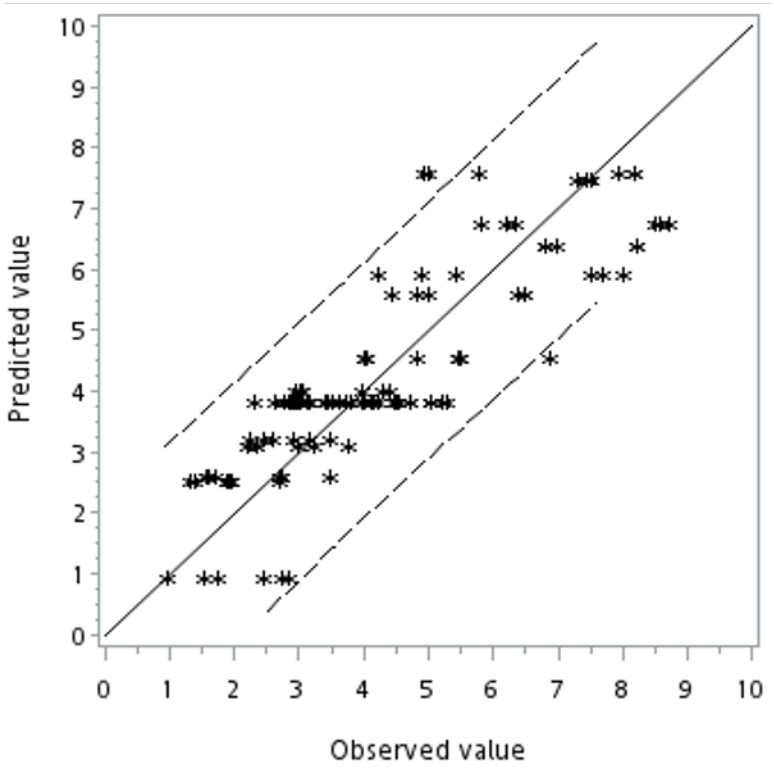
$$\text{Log}\,(N_o/N) = 65.2171 \left[\frac{P-200}{P+200}\right]^{1.4464} \left[\frac{C-0.05}{C+0.05}\right]^{1.0108} \left[\frac{T-6.0}{T+6.0}\right]^{0.7445}$$

Sum of squared error/uncorrected total: 175.9/ 2569.7

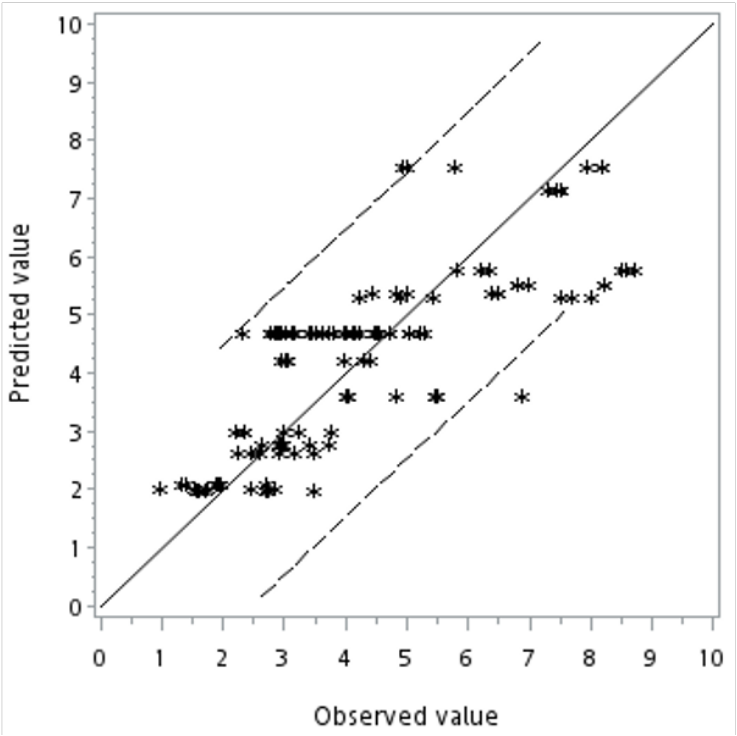
F value = 423.64, Pr > F (< 0.0001) (Eq. 2)

Model Performance: Prediction vs. Experiment data

(A) Linear Model (Eq. 1)



(B) Dimensionless Non-Linear Model (Eq. 2)



Experimental Validation of Predictive Models
(log reduction of E. coli O157:H7 and UPEC in ground chicken)

1

Run	Parameter			Log_{10} reduction (CFU/g) (or Lethality)			
				E. coli O157:H7		UPEC	
	Pressure (MPa)	TC (%)	Time (min)	Exp.[a]	Model: (Eq. 1 / 2)	Exp.[a]	Model: (Eq. 1 / 2)
1	330	0.40	22	3.79±0.24	3.77/4.27	4.49±0.35	3.77/4.27
2	370	0.30	18	4.02±0.38	3.63/4.67	4.52±0.22	3.63/4.67
3	270	0.20	30	1.86±0.23	na/1.75	2.12±0.45	na/1.75
4[#]	450	0.10	10	3.52±0.34	na/1.74	4.03±0.46	na/1.74

Initial populations of *E. coli* O157:H7 and UPEC on ground chicken: ca. 8.6 and 8.4 log CFU/g, respectively.
The detection limit was 1.0 log CFU/g.
Exp: experiment data
[a] Values represent mean ± standard deviation.
[#] Pressure at 450MPa (far over the 400 MPa range) may not be applied with the nonlinear models.
TC: trans-cinnamaldehyde.
na: not applicable (i.e. parameters outside the CCD range may not use the linear model)

Reference: Shiowshuh Sheen, Chi-Yun Huang, Rommel Ramos, Shih-Yung Chien, O. Joseph Scullen, and Christopher Sommers. (2018). Lethality prediction for Escherichia coli O157:H7 and Uropathogenic E. coli in ground chicken treated with high pressure processing and trans-cinnamaldehyde. J Food Sci. (in press). doi: 10.1111/1750-3841.14059

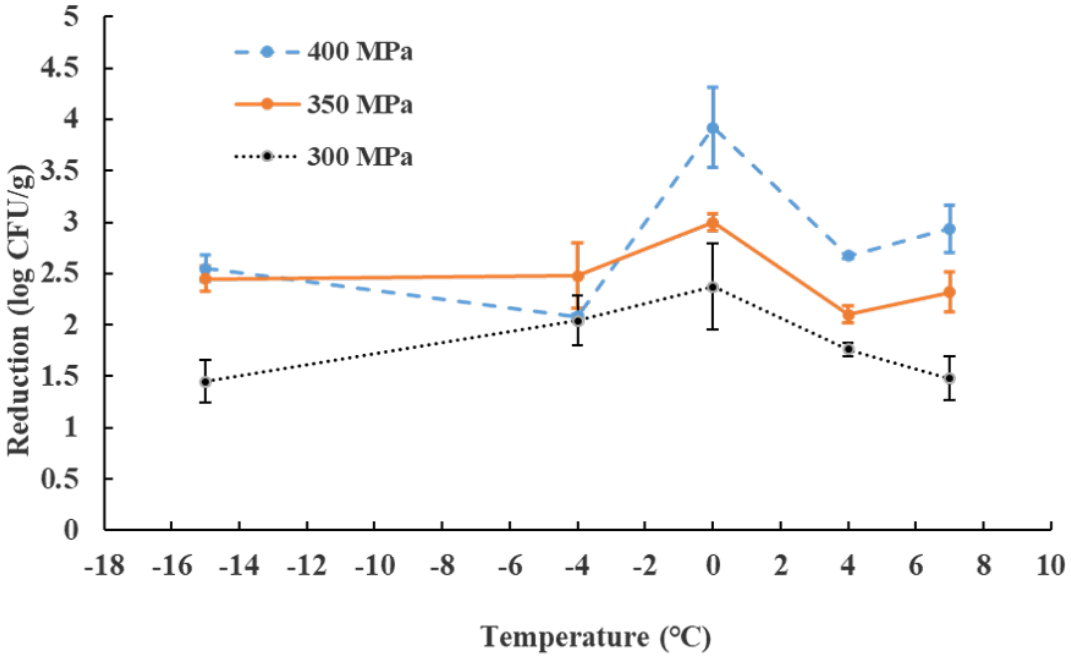
Variables and Levels used in a 4-factor 2-level Full Factorial Design

Factor	Level	Low level	Middle level	High level
	Unit	-1	0	+1
Temperature	°C	-15	-5	4
Pressure	MPa	250	300	350
Time	minute	10	15	20
AITC Concentration	% (w/w)	0.05	0.10	0.15

Table. Log reductions of *E. coli* O157:H7 on ground chicken meat after HPP treatments according to the four-parameter, two-level factorial design. (16 design + 2 center points - No. 1 and 18)

Trail No.	Temperature Celsius (level)	Pressure MPA (level)	Time minute (level)	AITC Concentration % % (w/w) (level)	Inactivation Log No - Log N E.coli:O157:H7
1	-5 (0)	300 (0)	15 (0)	0.10 (0)	3.82 ± 0.31
2	-15 (-1)	250 (-1)	10 (-1)	0.05 (-1)	1.34 ± 0.08
3	4 (+1)	250 (-1)	10 (-1)	0.05 (-1)	0.85 ± 0.08
4	-15 (-1)	350 (+1)	10 (-1)	0.05 (-1)	2.72 ± 0.29
5	4 (+1)	350 (+1)	10 (-1)	0.05 (-1)	2.26 ± 0.11
6	-15 (-1)	250 (-1)	20 (+1)	0.05 (-1)	2.19 ± 0.1
7	4 (+1)	250 (-1)	20 (+1)	0.05 (-1)	1.60 ± 0.02
8	-15 (-1)	350 (+1)	20 (+1)	0.05 (-1)	6.38 ± 0.26
9	4 (+1)	350 (+1)	20 (+1)	0.05 (-1)	2.88 ± 0.12
10	-15 (-1)	250 (-1)	10 (-1)	0.15 (+1)	2.43 ± 0.21
11	4 (+1)	250 (-1)	10 (-1)	0.15 (+1)	2.00 ± 0.17
12	-15 (-1)	350 (+1)	10 (-1)	0.15 (+1)	5.79 ± 0.05
13	4 (+1)	350 (+1)	10 (-1)	0.15 (+1)	6.70 ± 0.83
14	-15 (-1)	250 (-1)	20 (+1)	0.15 (+1)	5.41 ± 0.42
15	4 (+1)	250 (-1)	20 (+1)	0.15 (+1)	5.85 ± 0.67
16	-15 (-1)	350 (+1)	20 (+1)	0.15 (+1)	7.18 ± 0.04
17	4 (+1)	350 (+1)	20 (+1)	0.15 (+1)	7.25 ± 0.09
18	-5 (0)	300 (0)	15 (0)	0.10 (0)	3.20 ± 0.15

Inactivation of *E. coli* O157:H7 under HPP
Affected by operation temperature (-15 to 7°C)



Huang C-Y, Sheen S, Sommers C and Sheen L-Y (2018). Modeling the Survival of Escherichia coli O157:H7 Under Hydrostatic Pressure, Process Temperature, Time and Allyl Isothiocyanate Stresses in Ground Chicken Meat. Front. Microbiol. 9:1871. doi: 10.3389/fmicb.2018.01871

Inactivation modeling for *E. coli* O157:H7 under Stresses of high pressure (*p*), process temperature (*T*), time (*t*) and AITC dose (*C*)

Linear model:

$$Y = 6.19509 - 0.07290 \cdot P - 0.81711 \cdot C + 0.25242 \cdot t + 0.03140 \cdot T + 0.07450 \cdot P \cdot C - 0.00055 \cdot P \cdot t - 0.00025 \cdot P \cdot T + 0.72167 \cdot C \cdot t + 0.79386 \cdot C \cdot T - 0.00411 \cdot t \cdot T + 0.00016 \cdot P^2$$

$R^2 = 0.90$

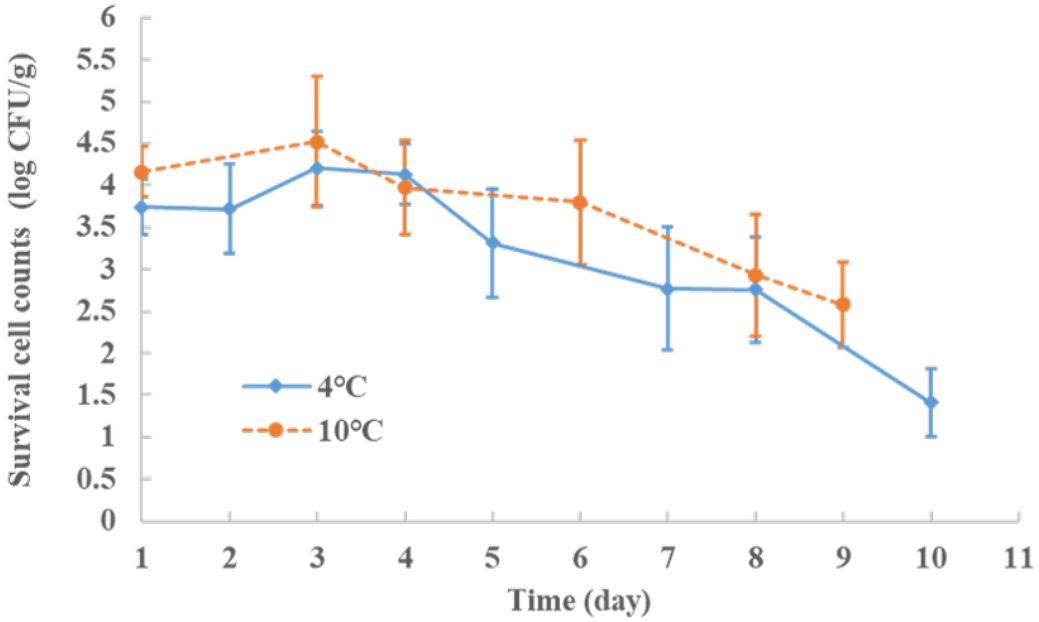
Non-linear model:

$$Z = 29.5243 \left[\frac{(P - 200)}{(P + 200)}\right]^{0.6417} \left[\frac{(C - 0.04)}{(C + 0.04)}\right]^{0.4005} \left[\frac{(t - 5.0)}{(t + 5.0)}\right]^{0.6544} \left[\frac{20 - T}{20 + T}\right]^{0.0441}$$

F value = 159.72; Pr > *F* (< 0.0001);
Sum of Squares Error and Sum of Squares Uncorrected Total are 61.2894 and 1060.2

Huang C-Y, Sheen S, Sommers C and Sheen L-Y (2018). Modeling the Survival of Escherichia coli O157:H7 Under Hydrostatic Pressure, Process Temperature, Time and Allyl Isothiocyanate Stresses in Ground Chicken Meat. Front. Microbiol. 9:1871. doi: 10.3389/fmicb.2018.01871

Survival behavior of *E. coli* O157:H7 stored at 4 or 10°C (300MPa, 15 min, process temp @ 4°C and 0.12% AITC dose)



The initial inoculum counts of the *E. coli* O157:H7 at 8.0 log CFU/g level

Huang C-Y, Sheen S, Sommers C and Sheen L-Y (2018). Modeling the Survival of Escherichia coli O157:H7 Under Hydrostatic Pressure, Process Temperature, Time and Allyl Isothiocyanate Stresses in Ground Chicken Meat.
Front. Microbiol. 9:1871. doi: 10.3389/fmicb.2018.01871

Developed Models for STEC O157:H7 in Ground Chicken Meat Subject to Hydrostatic Pressure (P), Process time (t), Allyl Isothiocyanate (C_1) and trans-Cinnamaldehyde (C_2) Stresses

(Confidential – to be published)

STEC O157:H7 (log CFU/g) reduction (S_1): Linear model

$$S_1 = -14.77432 + 0.13764 \cdot P - 0.37356 \cdot t - 70.20486 \cdot C_1 - 31.66458 \cdot C_2$$

$$+ 0.00124 \cdot P \cdot t + 0.24903 \cdot P \cdot C_1 + 0.11575 \cdot P \cdot C_2 + 1.24306 \cdot t \cdot C_1$$

$$+ 0.56583 \cdot t \cdot C_2 - 0.00029 \cdot P^2$$

$R^2 = 0.97$

STEC O157:H7 (log CFU/g) reduction (S_2): Non-linear dimensionless model

$$S_2 = 129.9 \left[\frac{P - 175}{P + 175}\right]^{1.6652} \left[\frac{t - 5}{t + 5}\right]^{0.8987} \left[\frac{C_1 - 0.01}{C_1 + 0.01}\right]^{0.9586} \left[\frac{C_2 - 0.07}{C_2 + 0.07}\right]^{0.3562}$$

Developed Models for STEC O157:H7 in Ground Chicken Meat Subject to Hydrostatic Pressure (P), Process time (t), Allyl Isothiocyanate (C_1) and trans-Cinnamaldehyde (C_2) Stresses
(Confidential – to be published) (2)

UPEC (log CFU/g) reduction (U_1): Linear model

$$U_1 = 7.81531 - 0.08524 \cdot P + 0.1795 \cdot t - 12.8125 \cdot C_1 - 2.79167 \cdot C_2$$

$$+ 0.00069 \cdot P \cdot t + 0.17528 \cdot P \cdot C_1 + 0.08767 \cdot P \cdot C_2 - 1.44444 \cdot t \cdot C_1$$

$$- 0.85167 \cdot t \cdot C_2 + 0.00016 \cdot P^2$$

$$R^2 = 0.93$$

UPEC (log CFU/g) reduction (U_2): Non-linear dimensionless model

$$U_2 = 44.7718 \left[\frac{P - 175}{P + 175}\right]^{1.0376} \left[\frac{t - 5}{t + 5}\right]^{0.5131} \left[\frac{C_1 - 0.01}{C_1 + 0.01}\right]^{0.3371} \left[\frac{C_2 - 0.07}{C_2 + 0.07}\right]^{0.1621}$$

Project progress in line with the set milestones (with models developed and validated)

HPP – potentially higher operation cost and food quality damage issues; (when used alone)

Antimicrobials: natural food grade (GRAS) compounds or chemicals (weak or little impact alone)

- **Thymol** and **HPP:** inactivated *E. coli* (O157:H7 and UPEC) on fresh ground meats. Frontiers in Microbiology, 2016 (Milestone 12 month)
- **Citral** and **HPP:** Food Control, 2017 (Milestone 24 month)
- **Trans-cinnamaldehyde** and **HPP**: J. Food Science, 2018 (Milestone 36 month)
- **Allyl Isothiocyanate** and **HPP**: Frontiers in Microbiology, 2018 (Milestone 36 month)
- **Multiple (Citral** & **Geraniol)** and **HPP:** Food and Bioprocess Technology, 2018 (Milestone 48 month)
- **Multiple (Allyl Isothiocyanate** & **Trans-cinnamaldehyde)** and **HPP:** in progress, 2019 (Milestone 48 month)

Conclusions

1. **HPP and properly selected antimicrobials may significantly enhance the pathogenic *E. coli* inactivation with lower hydrostatic pressure levels**
2. ***E. coli* O157:H7 and UPEC may show different resistance against intervention means**
3. **UPEC was found more sensitive to HPP and antimicrobial stresses than *E. coli* O157:H7 (in this report)**
4. **Models to predict the lethality were developed and validated (in ground meats)**
5. **Models may assist the risk assessment**

Challenges

Process scale-up and optimization to achieve targeted lethality
in certain foods may need considerations in:

1. HPP operation parameters and antimicrobials

2. Texture concerns (color and mouth-feel)

3. Operation cost (may be offset by consumer acceptance)

List of publications (including in-preparation status)

Hsu H-Y., **Sheen* S**., Sites J., Cassidy J., Scullen J.O. Sommers C. (2015). Effect of high pressure processing impact on the survival of Shiga toxin-producing *Escherichia coli* ("Big Six" and O157) in ground beef. Food Microbiology 48:1-7 (*Co-principal and Correspondent author)

Chien S-Y., **Sheen* S**., Sommers C.H., Sheen L-Y. (2016). Modeling the inactivation of intestinal pathogenic *Escherichia coli* O157:H7 and uropathogenic *E. coli* in ground chicken by high pressure processing and thymol. Frontiers in Microbiology, 7:920. Doi: 10.3389/fmicb.2016.00920 (*Co-principal and Correspondent author)

Chien S-Y., **Sheen* S**., Sommers C.H., Sheen L-Y. (2017). Modeling the inactivation of *Escherichia coli* O157:H7 and uropathogenic *E. coli* in ground beef by high pressure processing and citral. Food Control, 73:672-680. Doi: 10.1016/foodcont.2016.09.017 (*Co-principal and Correspondent author)

Sheen S., Huang C-H., Ramos R., Chien S-Y., Scullen O.J., Sommers C. (2018). Lethality prediction for *Escherichia coli* O157:H7 and uropathogenic *E. coli* in ground chicken treated with high pressure processing and trans-cinnamaldehyde. J Food Science. 83(3)740-749. DOI:10.1111/1750-3841.14059

Huang C-Y., **Sheen* S**., Sommers, C.H., Sheen L-Y. (2018). Modeling the survival of *Escherichia coli* O157:H7 under hydrostatic pressure, process temperature, time and allyl isothiocyanate stresses in ground chicken. Frontiers in Microbiology 9:1871, Doi: 10.3389/fmicb.2018.01871 (*Co-principal and Correspondence author)

Chien S-Y., **Sheen* S**., Sommers, C.H., Sheen L-Y. (2018). Combination effect of high pressure processing and essential oil (Melissa officinalis extract) or their constituents for the inactivation of *Escherichia coli* in ground beef. (2018). Food and Bioprocess Technology. (*Co-principal and Correspondence author)

Chuang S., **Sheen* S**., Sommers C.H., Zhou S., Sheen L-Y. (2019). Survival Evaluation for Salmonella and Listeria monocytogenes in Ground Chicken Meat Subject to High Hydrostatic Pressure and Carvacrol Using Selective and Nonselective Media (*Co-principal and Correspondence author; submitted to J. Food Protection).

Chuang S., **Sheen* S**., Sommers C.H., Sheen L-Y (2019). Modeling the Survival Behavior of *Escherichia coli* O157:H7 and Uropathogenic *E. coli* in Ground Chicken Meat Subject to Hydrostatic Pressure, Allyl Isothiocyanate and trans-Cinnamaldehyde Stresses (*Co-principal and Correspondence author) (In Preparation)

Staff/collaborators with contribution

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Mr. Rommel Ramos

Mr. Butch Scullen

Mr. Shih-Yung Chien, NTU – Visiting Scientist

Ms. Chi-Yun Huang, NTU – Visiting Scientist

Mr. Shih-Yu Chuang, NTU – Visiting Scientist