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.

Collaborators and Coauthors

- James R. Johnson, MD. Urologist, Dept. of Veterans Affairs and Univ. Minnesota.
- Dr. Lee-Yan Sheen & Students, National Taiwan University.
- Dr. Pina Fratamico, Dr. Lihan Huang and Dr. David Needleman (ERRC)
- Our Staff & Students (Dr. Shiowshuh Sheen, Butch Scullen, Rommel Ramos, Students: Shannon Tilman, Kristy Parker)
- Our future collaborators from PA Dept. of Health, Univ. Penn, Penn State, FDA NARMS.
- Dr. William Mackay, Edinboro University of PA

Escherichia coli Types

- Commensal (harmless background microflora)

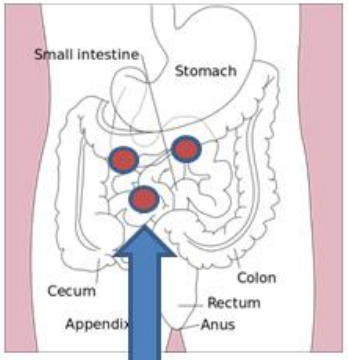
- Intestinal Pathogenic *E. coli* (iPEC)
  - STEC (B_1)
  - EHEC
  - VTEC

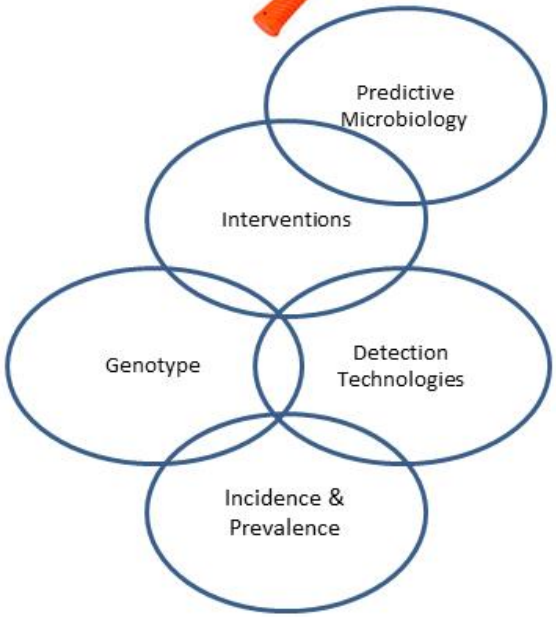
- Extraintestinal Pathogenic *E. coli* (B_2 *and D)*
  - Uropathogenic *E.coli (UPEC)*
  - Neonatal meningococcal *E. coli* (NMEC)
  - Avian pathogenic *E. coli* (APEC)
  - Sepsis-associated (SEPEC)

- Hybrids (Carry both iPEC and ExPEC Virulence Factors)

Path to Illness (ExPEC)

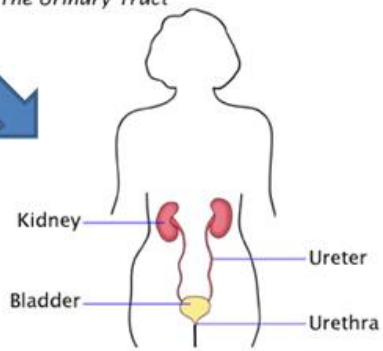
Reduce Risk





Ulcerative colitis
IBD/CD

The Urinary Tract



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 5th 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.

ExPEC and Ulcerative Colitis

Ca. 1.1 million cases in the US and EU annually. $3 billion.

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.

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

Urinary Tract Infections

- ca. >10 million cases in the US annually
- >130-175 million cases world wide annually
- Ca. 85% caused by *E. coli*
- *ca. 5% each caused by Staphylococcus saprophyticus or Klebsiella pneumoniae*
- 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

- 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?

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

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zzzzProtectins 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

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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

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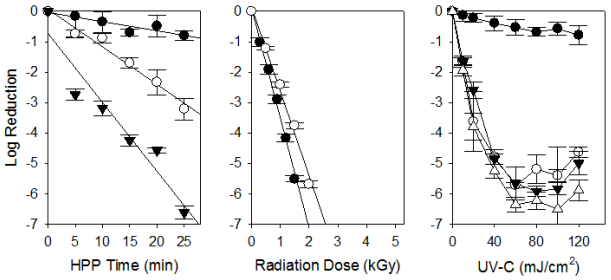
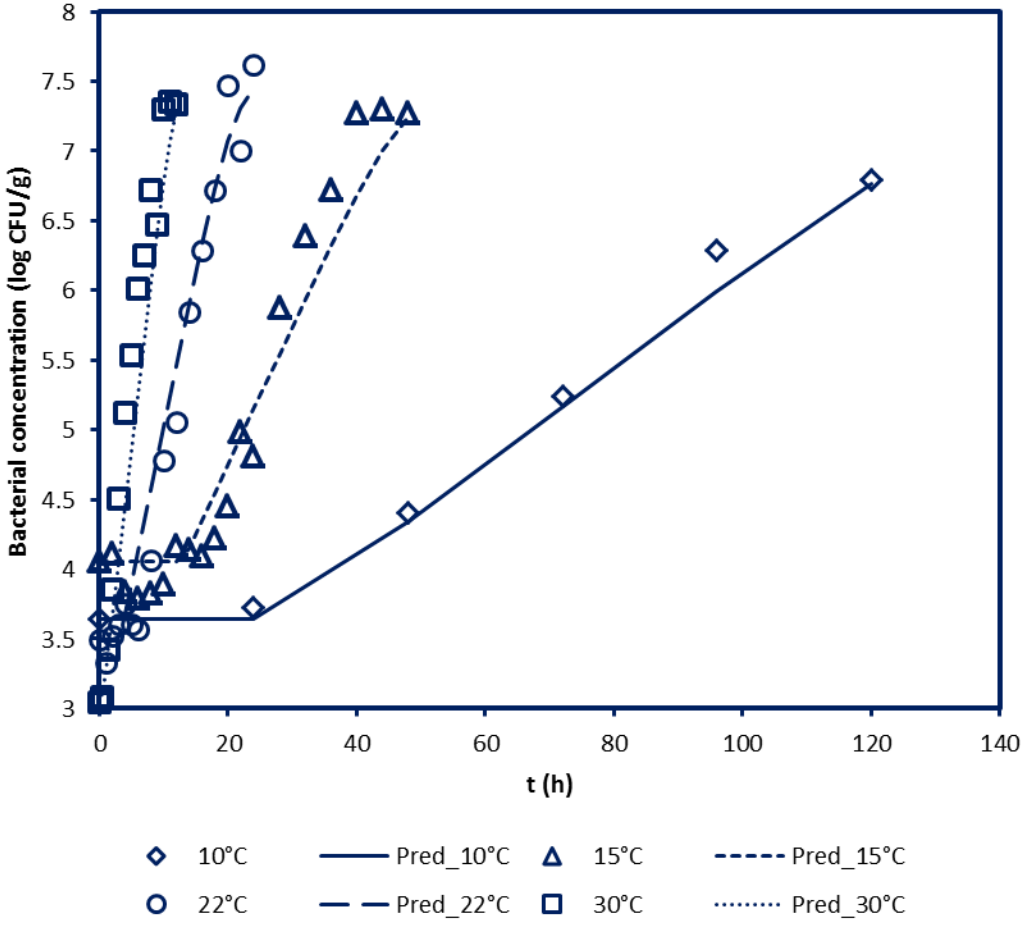
Inactivation of UPEC (Nonthermal)

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/cm^2
	HDPP	11.4 (±0.47) mJ/cm^2
	HDPE	12.9 (±0.59) mJ/cm^2



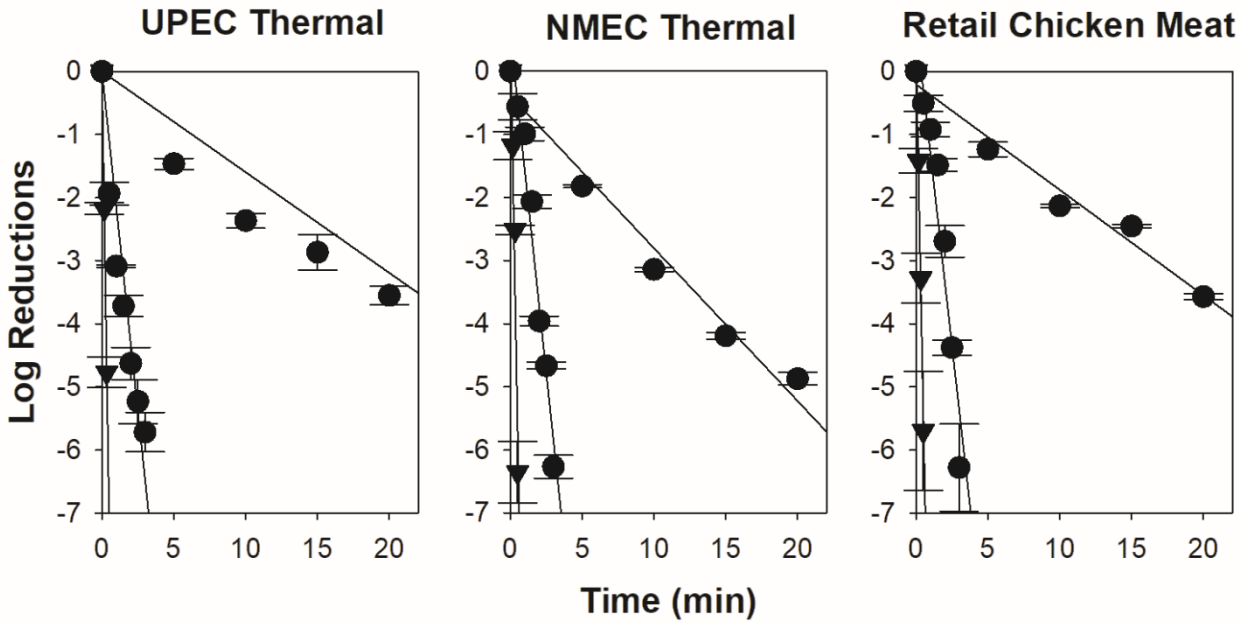
Sommers C, Scullen O, Sheen S (2016) Inactivation of Uropathogenic Escherichia coli in Ground Chicken Meat Using High Pressure Processing and Gamma Radiation, and in Purge and Chicken Meat Surfaces by Ultraviolet Light. Front. Microbiol. 7:413. doi: 10.3389/fmicb.2016.00413

UPEC Growth Curves and Model for Ground Chicken Meat



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.

ExPEC Thermal Inactivation (unpublished)



ExPEC Type	Temp	D-10 (min)	*z*-value
UPEC	55 ºC	7.34	4.69 ºC
	60 ºC	0.56	
	65 ºC	0.06	
NMEC	55 ºC	4.13	5.89 ºC
	60 ºC	0.50	
	65 ºC	0.08	
Retail Chicken	55 ºC	5.98	5.53 ºC
	60 ºC	0.49	
	65 ºC	0.09	

ExPEC Isolated From Chicken Skin (Unpublished)

Sample	O:H	PG	ST1	ST2	Genome	AR	ExPEC Type	Virulence Factors
B7S3	102:H6	B2	ST115	ST45	5738343	aac(3)-Via, aadA1, sul1,sul2	iutA kpsMII	astA, iha, eilA, iss, cba, cma, air
B7S10	102:H6	B2	ST115	ST45	5736502	aac(3)-Via, aadA1, sul1,sul2	iutA kpsMII	astA, iha, eilA, iss, cba, cma
B7S19	102:H6	B2	ST115	ST45	5732502	aac(3)-Via, aadA1, sul1,sul2	iutA kpsMII	astA, iha, eilA, iss, cba, cma, air
B7S21	102:H6	B2	ST115	ST45	5733839	aac(3)-Via, aadA1, sul1,sul2	iutA kpsMII	astA, iha, eilA, iss, cba, cma, air
B7S22	102:H6	B2	ST115	ST45	5732601	aac(3)-Via, aadA1, sul1,sul2	iutA kpsMII	astA, iha, eilA, iss, cba, cma, air
B7S29	102:H6	B2	ST115	ST45	5737478	aac(3)-Via, aadA1, sul1,sul2	iutA kpsMII	astA, iha, eilA, iss, cba, cma, air
B7S30	102:H6	B2	ST115	ST45	5730122	aac(3)-Via, aadA1, sul1,sul2	iutA kpsMII	astA, iha, eilA, iss, cba, cma, air
B7S31	102:H6	B2	ST115	ST45	5730122	aac(3)-Via, aadA1, sul1,sul2	iutA kpsMII	astA, iha, eilA, iss, cba, cma, air
B7S38	102:H6	B2	ST115	ST45	5736335	aac(3)-Via, aadA1, sul1,sul2	iutA kpsMII	astA, iha, eilA, iss, cba, cma, air
B8S18	102:H6	B2	ST770	Unknown	5547966	None	iutA kpsMII	tsh, gad, iroN, iha, iss, eilA, mchF
B8S55	102:H6	B2	ST7284	ST45	5701011	sul2	iutA kpsMII	astA, iha, eilA, iss, cma, gad, air
B8S56	102:H6	B2	ST7284	ST45	5695622	sul2	iutA kpsMII	astA, air, iss, iha, eilA, cma
B8S59	O11:H25	B2	ST1674	Unknown	5417305	strA, strB, tet(B)	iutA kpsMII	astA, iss, air, mchF, lpfA, iroN

General Linear Regression Model

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

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

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

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

Model for Microbial Survival or inactivation

- General Expression of a Dimensionless Non-linear model

  - Lethality (Sheen's model):

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

k, mi: to be determined;

$X_{min/max}$: parameter at min or max effective level

Escherichia coli O157:H7 and UPEC inactivation on ground chicken impacted by HPP, process time, and trans-cinnamaldehyde dose

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)

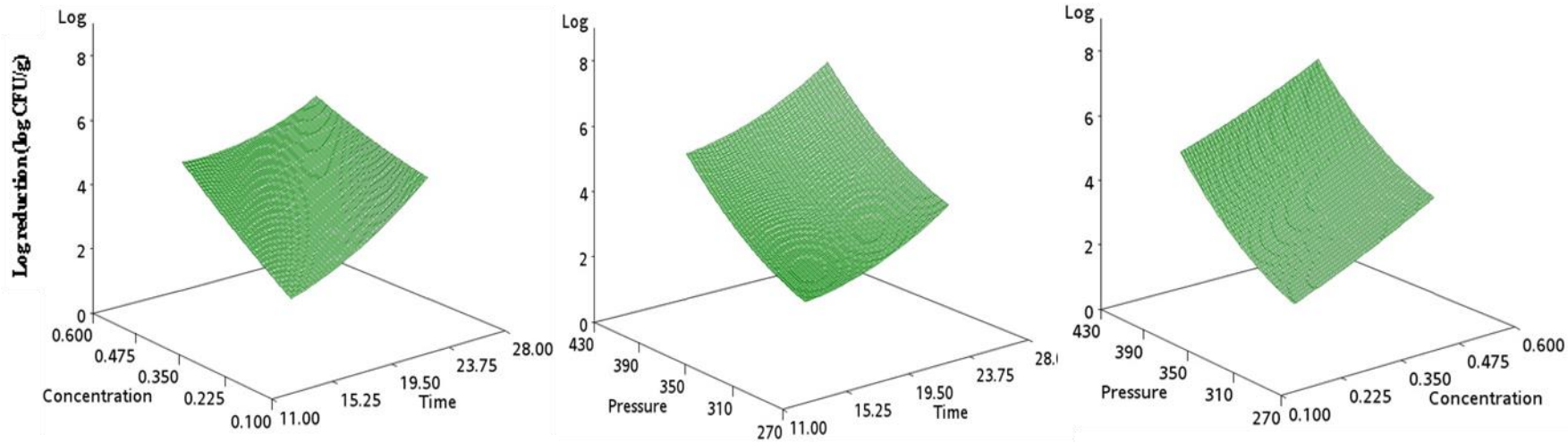
Dimensionless nonlinear model (Sheen's model):

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

Sum of squared error/uncorrected total: 175.9/ 2569.7

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

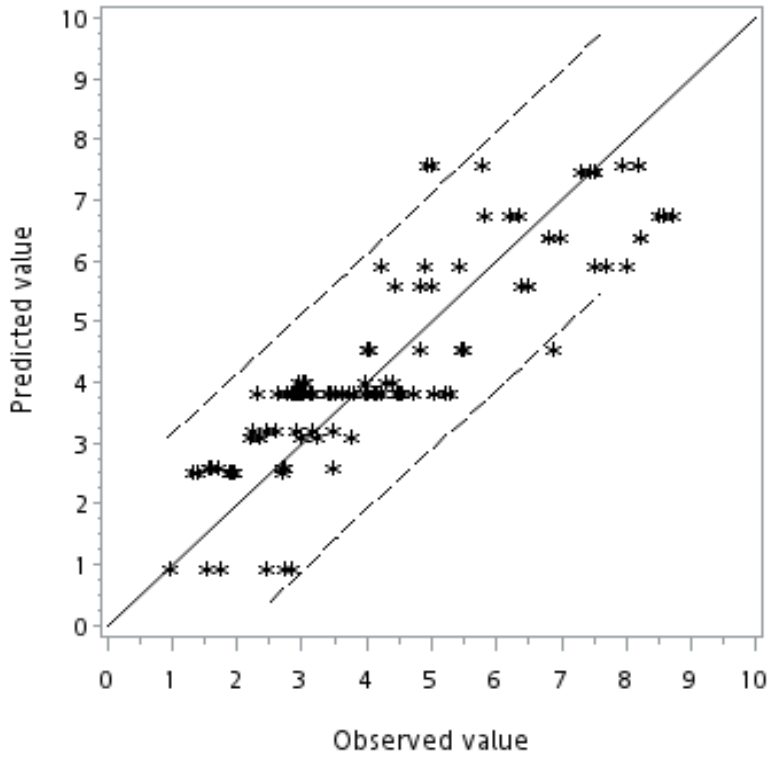
Response surface 3D plots showing the effect of (a) concentration and time, P= 350MPa (b) pressure and time, C=0.35% dose and (c) pressure and concentration T=20min, (combined data of *E. coli* O157:H7 and UPEC) – may be used for process optimization



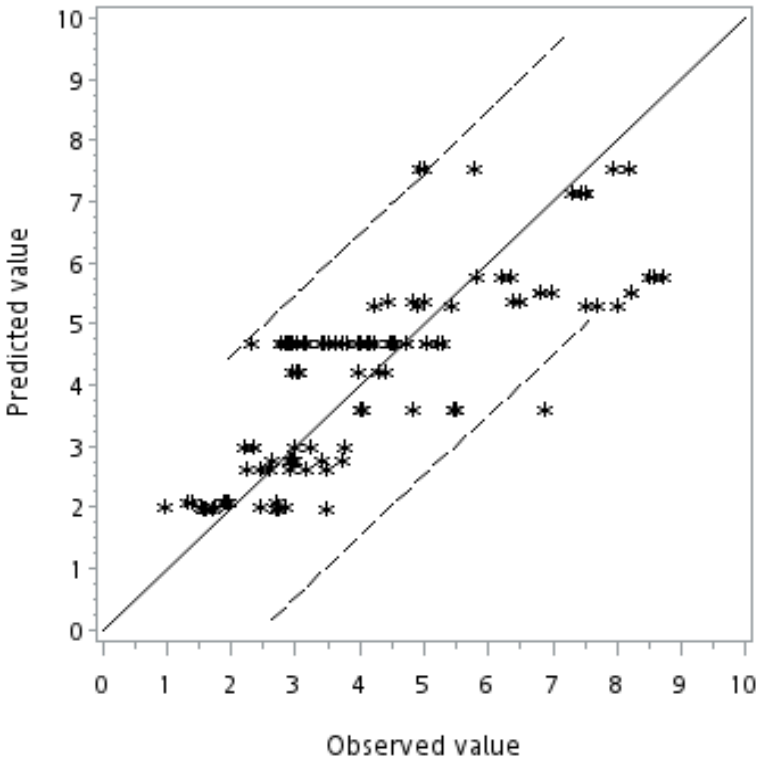
(a) (b) (c)

Model Performance: Prediction vs. Experiment data

(A) Linear Model (Eq.1)



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



Experimental Validation of Predictive Models

1 (log reduction of *E. coli* O157:H7 and UPEC in ground chicken)

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