ILSI North America Sponsored Research on Mitigation of Salmonella in Spices

ASTA
Annual Meeting 2017

ILSI™ North America

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Collaboration

We work with industry, government and academic scientists to conduct research.

Scientific Integrity

We commit to publishing our results no matter what the outcome.

Transparency

We are committed to making our research methods and data available to the scientific community

Public Benefit

Our projects must offer benefit to the health of the public

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ILSI North America

ILSI branches worldwide…

About ILSI North America

www.ilsina.org

Current areas of work:

- Bioactives
- Caffeine
- Carbohydrates
- Scientific Integrity
- Dietary Lipids
- Balancing Food & Activity for Health
- Fortification
- **Food Microbiology**
- Food and Chemical Safety
- Low-Calorie Sweeteners
- Gut Microbiome
- Packaging
- Protein
- Sodium

Stay up to date on all our work:

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Technical Committee on Food Microbiology

The Committee is committed to proactively improving the understanding and control of microbial food safety hazards to enable scientifically informed decision making.

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New Committee Research on Pathogens in Low Water Activity Foods

"Survival and Inactivation of Bacterial and Viral Pathogens in Model Low-Moisture Foods"

University of Guelph and Health Canada

NC State University

Food Matrices: dried apples, raisins, dried strawberries, pistachios, corn flake cereal, and chocolate liquor

"*Listeria monocytogenes* Thermal Resistance in Low Moisture Foods: Role of Water Activity or Food Matrix"

Washington State University

Food Matrices: skim milk powder, almond meal/flour, and cocoa powder

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New Committee Research on Sampling

Committee is releasing a new RFP on
Sampling and Sample Prep for Microbiological Testing
Summer 2017

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Identification of Spice Research 2013 Research Roundtable with Key Federal Agencies

- Spices and seasoning categories were among the top three categories in the FDA Reportable Food Registry submissions for *Salmonella* contamination in foods between 2009 and 2011.
- The most current version of the FDA Reportable Food Registry, a 5 year overview from 2009 to 2014, indicates that spices and seasonings accounted for the majority of *Salmonella*-related reports in 2014.
- The presence of *Salmonella* is of particular concern when spices are used in ready-to-eat foods, added post pathogen-reduction step or post processing, such as in cereals, crackers, salad dressings

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Spice Research Projects

- "Protocol and Surrogate Validation for the Inactivation of *Salmonella* on Spices"- *Monica Ponder, Virginia Tech*

  Grant Award $207,688

- "Correlation of Surrogate Bacteria and *Salmonellae* for Validation of Spice/Herb Pathogen Reduction Processes"- *Gary Acuff, Texas A & M University*

  Grant Award $142,552

Total Grant Awards

$350,210

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Collaboration with ASTA

- ILSI North America Food Microbiology Committee collaborated with ASTA in development of the RFP for spices
- Awarded two spice research grants in June 2013
- ASTA provided:
  - $75,000 in an unrestricted grant to ILSI North America
  - Provided Spices in-kind for the research
  - Coordination for visit to Cosmed facility, Dec 2013
  - Coordination for ethylene oxide (EtO) studies conducted at Cosmed facility, June 2016

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ILSI North America Spice Research Projects

Dr. Ponder (Virginia Tech)

Dr. Acuff (Texas A&M and Iowa State University)

Salmonella: variety of strains
Salmonella Surrogate Strain: *Enterococcus faecium* NRRL B-2354

Black Peppercorn

Cumin Seed

Black Peppercorn

Cumin Seed

Oregano

Onion Powder

Ethylene Oxide (EtO)

Dry Steam

Irradiation

Irradiation

Sensory Testing:
Color/Aroma/Volatiles/Qualitative Evaluation

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Bacterial Log reduction
90 % reduction of population

- $10{,}000{,}000 = 10^7 = \log 7$
- $1{,}000{,}000 = 10^6 = \log 6$
- $100{,}000 = 10^5 = \log 5$
- $10 = 10^1 = \log 1$

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Dr. Gary Acuff's Study:

Correlation of Surrogate Bacteria and *Salmonellae* for Validation of Spice/Herb Pathogen Reduction Processes

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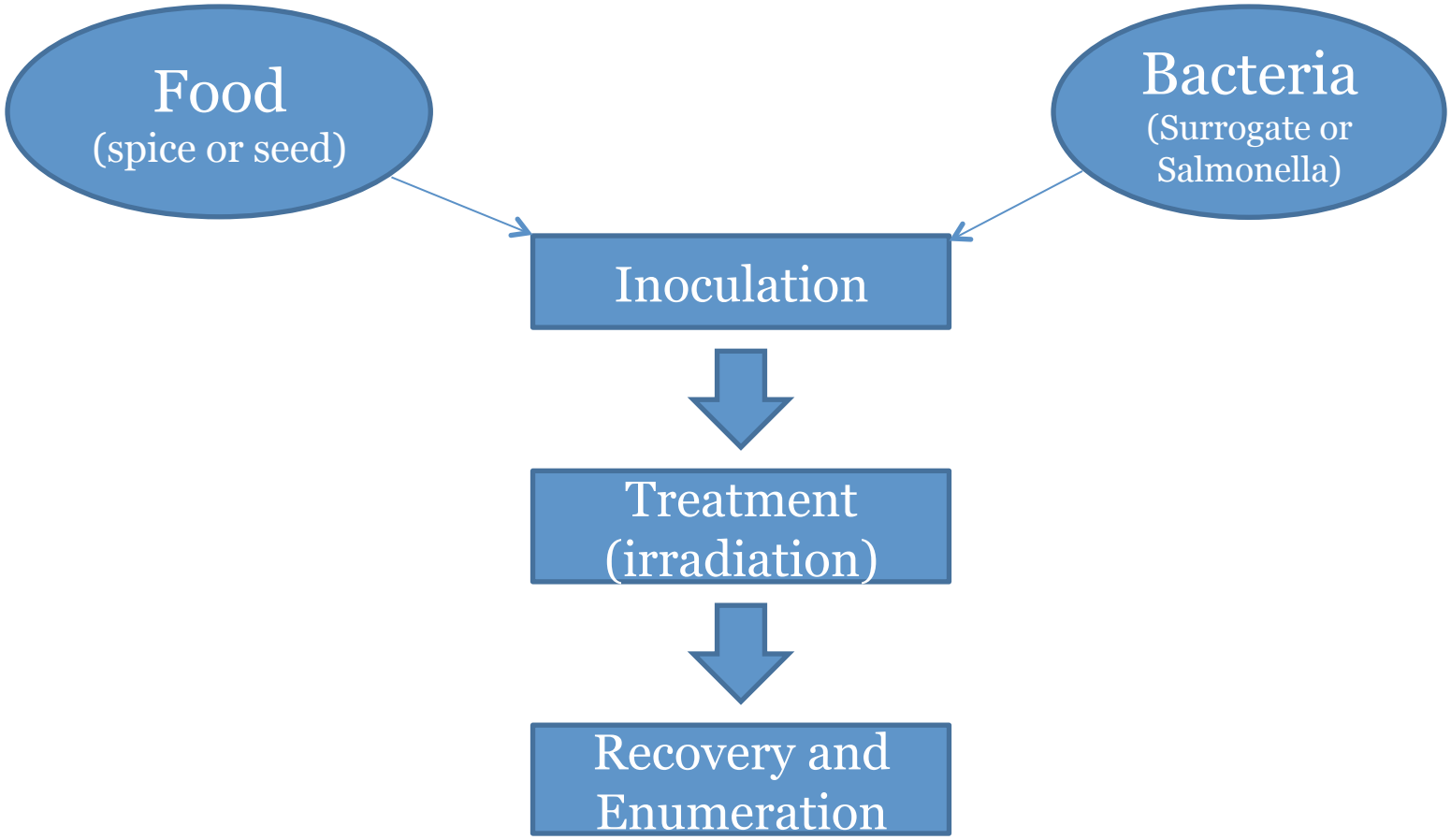
Steps to a Validation:



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Component of Process Validation: Inoculation Method

- *Salmonella* and *Enterococcus faecium NRRL B-2354 (E. faecium)* were dried separately on talc powder, then the talc powder was mixed with spices.
- Spice-related *Salmonella* strains were more resistant to desiccation than non-spice-related *Salmonella* strains.
  - **Recommendation:** a mixture of spice-related *Salmonella* strains should be used for in-laboratory challenge studies involving *Salmonella* in dry spices.
- Using talc powder as a dry inoculum for a dry herb (ex: oregano) was successful. Likely that the same method could be extended to other dried herbs.
  - **Recommendation:** dry inoculum with talc as a carrier can be used to inoculate dry spices, however always validate the recovery of the organism

Component of Process Validation: Enumeration of Surviving Target Bacteria after Treatment

- Overlay methodology used: cells are first plated onto non-selective media followed by a overlay of selective media

Component of Process Validation: Identification of Surrogate

- The stability of both bacteria was compared:
  - during storage of the spices,
  - during drying in the spices, and
  - when exposed to incremental doses of irradiation.
- The reductions in populations were comparable for both bacteria in all 3 situations

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Decimal reduction values kGy

	Salmonella	Surrogate, *E. faecium*
Onion powder	0.84	1.103
Dried oregano	1.16	1.10
Whole cumin seeds	1.57	1.62
Peppercorns	1.67	1.70

- Decimal reduction values is the dose (kGy) that reduces the population by 90%

- A higher D value indicates that a more severe treatment is required to reduce the same %age of the population. We want the surrogate to have equivalent or higher D value.

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Reduction of the surrogate, *E. Faecium*, equivalent to 3 or 5 log reduction of *Salmonella*

Spice	3 Log reduction *Salmonella*	5 Log reduction *Salmonella*
Onion Powder	2.28	3.81
Dried Oregano	3.16	5.27
Whole Cumin Seeds	2.92	4.86
Peppercorns	2.94	4.90

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Multiple Publications Expected from Acuff Team

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Dr. Monica Ponder's Study:

Protocol and Surrogate Validation for the Inactivation of *Salmonella* on Spices

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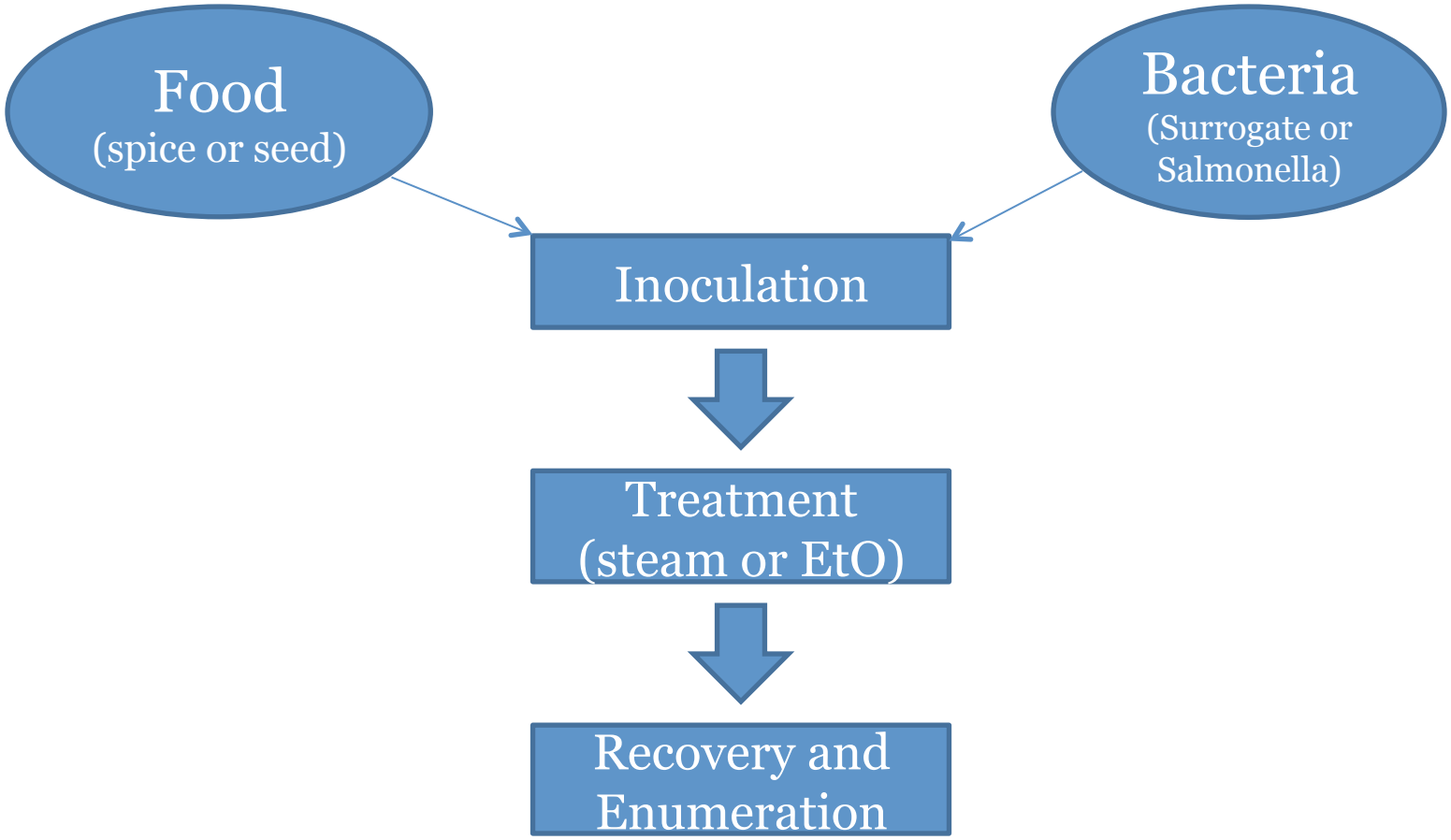
Steps to a Validation:



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Component of Process Validation: Inoculation Method

- Investigated inoculation preparation methods:
  - Modified wet inoculation:
  - Dry transfer method with sand particles
  - Immersion/Biofilm: *Salmonella* encased within a biofilm formed by the spice native microbiota. Resulted in populations that were highly stable over long-term storage.
    - Works best for whole peppercorns but is not recommended for cumin seeds (evidence that the seed swelled, internalizing the *Salmonella)*
- **Recommendation:** A modified wet inoculation procedure where the bacteria are grown on a TSA- plate, suspended in a small volume of liquid, which is used to coat the whole spices subsequent to drying. This methodology is reproducible.

Component of Process Validation: Recovery and Enumeration of Surviving Target Bacteria after Treatment

- Investigated several methods of recovery and enumeration to improve recovery in the presence of native microflora:
  - Without overlay of selective media, background flora can confound results.
  - Using only selective media does not allow for partially damaged cells to recover.
  - Overlay methodology used: cells are first plated onto non-selective media followed by a overlay of selective media (observed increase by 1 log on treated peppercorns, and increase 2 logs on treated cumin seeds)
- **Recommendation:** an overlay methodology enhances recovery of cells partially damaged by the treatment. These partially damaged cells may later recover and grow.

Component of Process Validation: Identification of Surrogate

- Surrogate Selected: *Enterococcus faecium* NRRL B-2354
- First Step: Identification of a non-pathogenic surrogate bacteria, of equivalent or higher process resistance compared to *Salmonella*, that can be used to validate process:
  - Depends on the substrate (spice type)
  - Packaging
  - Process
  - other
- EtO
- Vacuum-assisted Steam

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Component of Process Validation: Mitigation Process using EtO

- EtO fumigation resulted in:
  - significantly larger reductions in *Salmonella* population compared to *E. faecium* on whole black peppercorns
  - no significant difference in reduction on cumin seeds.
  - Thus *E. faecium* is suitable as an equivalent surrogate for validating thermal and gas parameters, load placement, arrangement and packaging configurations of whole black peppercorns and cumin seeds with EtO fumigation.
- **Recommendation:** Validates the use of *E. faecium* as an equivalent surrogate for inactivation of *Salmonella* inoculated on whole peppercorns and cumin seeds treated with EtO.

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Component of Process Validation: Mitigation Process using Vacuum- Assisted Steam

Vacuum- Assisted Steam Processing of inoculated spices resulted in:

- Lab Scale Method:
  - No significant difference in the reduction of *Salmonella* and *E. faecium* for peppercorns and cumin seeds, indicating that *E. faecium* may be considered an equivalent surrogate.
  - Identified 2 sets of minimum conditions, minimum times/temperatures for product processing, (165 °F & 185°F), allowing commercial processors to adjust time of process, etc. to achieve these targets.
- Commercial chamber Method:
  - No significant difference in reduction of S*almonella* and *E. faecium* on peppercorns
  - Less reduction of *Salmonella* than *E. faecium* on cumin seeds, which may indicate that E. faecium may not be a suitable surrogate on cumin seeds processed commercially if steam temperature and moisture conditions are not maintained at 165°F.

Recommendation: *E. faecium* was considered an equivalent surrogate for inactivation of *Salmonella* inoculated on peppercorns treated with steam, but not cumin seeds at the defined temperatures.

Component of Process Validation: Sensory

EtO	Steam	Irradiation
Whole Black Peppercorns: the treatment did not create detectable odor or appearance differences.	Whole Black Peppercorns: the treatment resulted in perceptible odor differences,	Whole Black Peppercorn, Whole Cumin seed and Oregano: Irradiation process did not alter quality of color or odor
Whole Cumin Seeds: the treatment did create differences in odor and appearance	Whole Cumin Seeds: treatment created only visual differences in seed color.	There was a difference in color for Onion Powder, and resulted in nearly complete loss of measured volatile compounds.
No data on onion powder or Oregano for EtO	No data on onion powder or Oregano for steam	

Conclusions of Dr. Ponder's Study

- Variability in packaging size and type, product type, process design and type, and quality requirements of suppliers, must be accounted for in any validation.
- *E. faecium* was investigated in this research, and found to be either of equivalent or higher process resistant compared to *Salmonella*, advantageous for its use in validating commercial processes.
- This eliminates the need to bring a pathogen into a manufacturing environment.
- Further research on fluid dynamics both within packages, considering packaging materials, and within chambers to optimize load placement, is needed to provide better predictions for different spice types and packaging materials.

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5 Publications from Ponder Team:

- "**Inoculation Preparation Affects Survival of *Salmonella enterica* on Whole Black Peppercorns and Cumin Seeds Stored at Low Water Activity**" (published March 2015 in the *Journal of Food Protection*)
- "**Processes to Preserve Spice and Herb Quality and Sensory Integrity During Pathogen Inactivation**" (published April 2017 in the *Journal of Food Science*)
- **"The Inactivation of *Salmonella enterica* and Surrogate *Enterococcus faecium* on Whole Black Peppercorns and Cumin Seeds Using Steam"**
- **"Improving Recovery of *Salmonella* spp. and *Enterococcus faecium* from Whole Black Peppercorns and Cumin Seeds Subjected to Steam"**
- **"Use of *Enterococcus faecium* as a Surrogate for *Salmonella enterica* on Whole Black Peppercorns and Cumin Seeds Subjected to Commercial Ethylene Oxide Processing Conditions"**

Importance to ASTA Members

- With FSMA, pathogen reduction steps must be adequately documented, for the specific process, product and package.
- Pathogen reduction technologies used to treat spices and herbs are Preventive Controls / Critical Control Points that can reduce microbiological risk to an acceptable level. Documenting the scientific basis for the pathogen reduction through experimentation is crucial, demonstrating effectiveness and consistency.
- The treatment of spices protects consumers and prevents recalls due to *Salmonella* contamination.

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Importance to ASTA Members

- Development of standardized validation protocols provides:
  - **Consumer protection**
  - Tools to assure the effectiveness of a variety of treatments
    - Inoculation procedures
    - Recovery and enumeration procedures
    - Identification of a surrogate
  - Reconditioning/rework processes acceptable to the FDA
  - Regulatory compliance for Preventive Controls

NOTE: Kill-step validated processes should be audited yearly or as directed by a qualified Food Safety Scientist to ensure that a particular process is consistently delivering a desired effect.

QUESTIONS?

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