Low Water-Activity Foods Safety Series: Part 3 of 4 – Microbiological Safety of Dried Spices

Moderator: Joshua Gurtler, USDA-ARS, United States

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Today's Moderator

Joshua Gurtler
USDA-ARS, United States

Joshua Gurtler is a Research Scientist at the USDA/ARS in Wyndmoor, PA, where he has worked for 14 years. Dr. Gurtler's current work involves interventions for the inactivation of foodborne pathogens in fresh produce, soil, water, compost, and dried foods. Joshua has published numerous peer-reviewed scientific manuscripts, 9 book chapters, is an editor of three books and author of one patent and one patent that is pending. He has served as a member or chair on several IAFP committees. Dr. Gurtler is a co-scientific editor for the Journal of Food Protection. He has delivered numerous scientific research presentations, including invited presentations, in the U.S., Canada, China and Korea. He resides with his wife and three children in Phoenixville, PA.

Today's Panelists

Alex Brandt, Ph.D.
Food Safety Net Services

Dr. Brandt joined FSNS in 2014 and serves as the Chief Science Officer. Dr. Brandt earned his B.S. (Summa Cum Laude) and M.S. from Texas A&M University in Food Science and Technology with an emphasis in Food Microbiology. He earned his Ph.D. from Texas Tech University in Animal Science with an emphasis in Food Microbiology. In his role as CSO, Dr. Brandt combines his knowledge of food processing operations with his expertise in microbiology and molecular biology to provide technical solutions for FSNS customers. This includes leading the FSNS Lab+ scientific team, which performs customized research studies such as process validations, challenge studies, method validation studies, and specialized testing services. Dr. Brandt also oversees the technical services team that provides technical guidance to customers on method validations, regulatory matters, and contamination troubleshooting. Dr. Brandt is a member of the International Association for Food Protection, AOAC International, and the American Meat Science Association in addition to serving on the Scientific Advisory Board for United Egg Producers.

Susanne Keller
FDA, United States

Dr. Keller received her B.S. degree in Microbiology and her M.S. and Ph.D. in Food Science from Michigan State University. She began her career with the NutraSweet Company (first as part of Searle, then as part of Monsanto) where she was involved with fermentation and product development (dairy). After seven years in industry, she joined the FDA initially working on pattern recognition during fermentation and mycotoxin production. Her subsequent work involved the survival of foodborne pathogens in juices and juice HACCP. For approximately the last ten years she has been involved with research related to the survival of pathogens on dry products such as spices and seeds. Dr. Keller is the author of publications in the general area of food microbiology and serves as a scientific expert on food microbiology for the FDA.

Foodborne pathogens and spices: An overview

Susanne Keller

FDA CFSAN

Spices

“Any aromatic (dried) vegetable substances in the whole, broken, or ground form, except for those substances which have been traditionally regarded as foods, whose significant function in food is seasoning rather than nutritional, and from which no portion of any volatile oil or other flavoring principle has been removed”

2013 FDA Draft Risk Profile: Pathogens and Filth in Spices

Spices

Includes additional dried plants such as dried vegetables used as seasonings

Dehydrated onion and garlic

Some “multi-purpose” dried plant parts
Sesame seeds: tahini
Mustard seeds: sprouts

FDA CFSAN

Spices: production/source

Primarily imported (US)

India
Indonesia

Only 5 spices produced in US in large quantities

dehydrated onion
dehydrated garlic
capsicum
mustard seed
sesame seed

FDA CFSAN

Spices: production/source

Over 100 different plants commonly used

Both annuals and perennials

All types of plant parts, some plants, more than one part and more than one spice

Example: mace and nutmeg
dill seed and dill leaf
coriander seed and leaf

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Spices: production/source

Leaves
oregano, basil, rosemary, cilantro, sage, thyme, tarragon, mint, bay leaf
Flowers
saffron, cloves
Stem/Bark
cinnamon
Root/rhizomes/bulb
ginger, turmeric, onion, garlic
Fruits/seeds
pepper, paprika, anise, nutmeg, coriander, sesame, vanilla, cardamom, mustard, annatto, fennel, dill, poppy, star anise, sumac, cumin, allspice, mace, nutmeg,

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Microbiology of “spice”

Dependent on environment/processing: expect everything

APC counts may be high or low

log 2 to log 9/g

Many types of pathogens have been found

Salmonella

Shigella

Bacillus spp. Including *B. cereus*

Clostridium perfringens

Cronobacter spp.

Staphylococcus aureus

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Microbiology of "spice"

Yeasts and Molds

typically 1-3 log/g

Common Species found:

Alternaria
Aspergillus
Botrytis
Fusarium
Penicillium
Pythium
Rhizopus

FDA CFSAN

Microbiology of “spice”

Variable levels of mycotoxins in spices
Bulent Kabak & Alan D. W. Dobson (2017) Mycotoxins in spices and herbs–An update, Critical Reviews in Food Science and Nutrition, 57:1, 18-34, DOI:10.1080/10408398.2013.772891

- **Affected by harvest/storage conditions**
- **Antimicrobial compounds affect mold growth and toxin production ex: cinnamic aldehyde and eugenol**

Cinnamon: 0.13-4.67 mg/Kg AFB$_1$
Al-Juraifani, A. A. (2011). Natural occurrence of fungi and aflatoxins of cinnamon in the Saudi Arabia. Afr. J. Food Sci. 5:460–465.

Clove powder: **AF at 29 mg/kg (India), OTA ,160 mg/kg, (Comoros) in 2009.**
RASFF notifications.

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Outbreaks associated with Spices

1973-2010: 14 reported illness outbreaks associated with spices

- Canada, Denmark, France, Germany, New Zealand, Norway, Serbia, United Kingdom, and the United States
- 1946 reported illnesses, 128 hospitalizations, 2 deaths
- Infants and children were the primary impacted population in 5 outbreaks

Causative agent

Salmonella enterica: 87% of reported illnesses.
Bacillus spp.: 13% of reported illnesses

More recently?
October, 2020, raw onion (not dried) S. Newport

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Recalls associated with contaminated spices

Reportable Food Registry, Fifth annual report 2015:

Salmonella in “Spices and seasonings” category:

16/17: year 1, 9-9-2009 to 9-7-10

23/25: year 2, 9-8-2010 to 9-7-11

5/8: year 3, 9-8-11 to 9-7-2012

10/12: year 4, 9-8-12 to 9-7-2013

11/12: year 5, 9-8-2013 to 9-7-2014

19% of total RFR entries for all 5 years

Ginger powder, May 1, 2017

Tarragon, Oct 16, 2017

Mixed spices (cumin, pepper) Nov 17, 2017

Chili kit, Feb 9, 2018

Whole Fennel Seed, July 22, 2019

Curry powder, May 21, 2020

Multiple spices, Oct 12, 2020

Parsley, Oct 13, 2020

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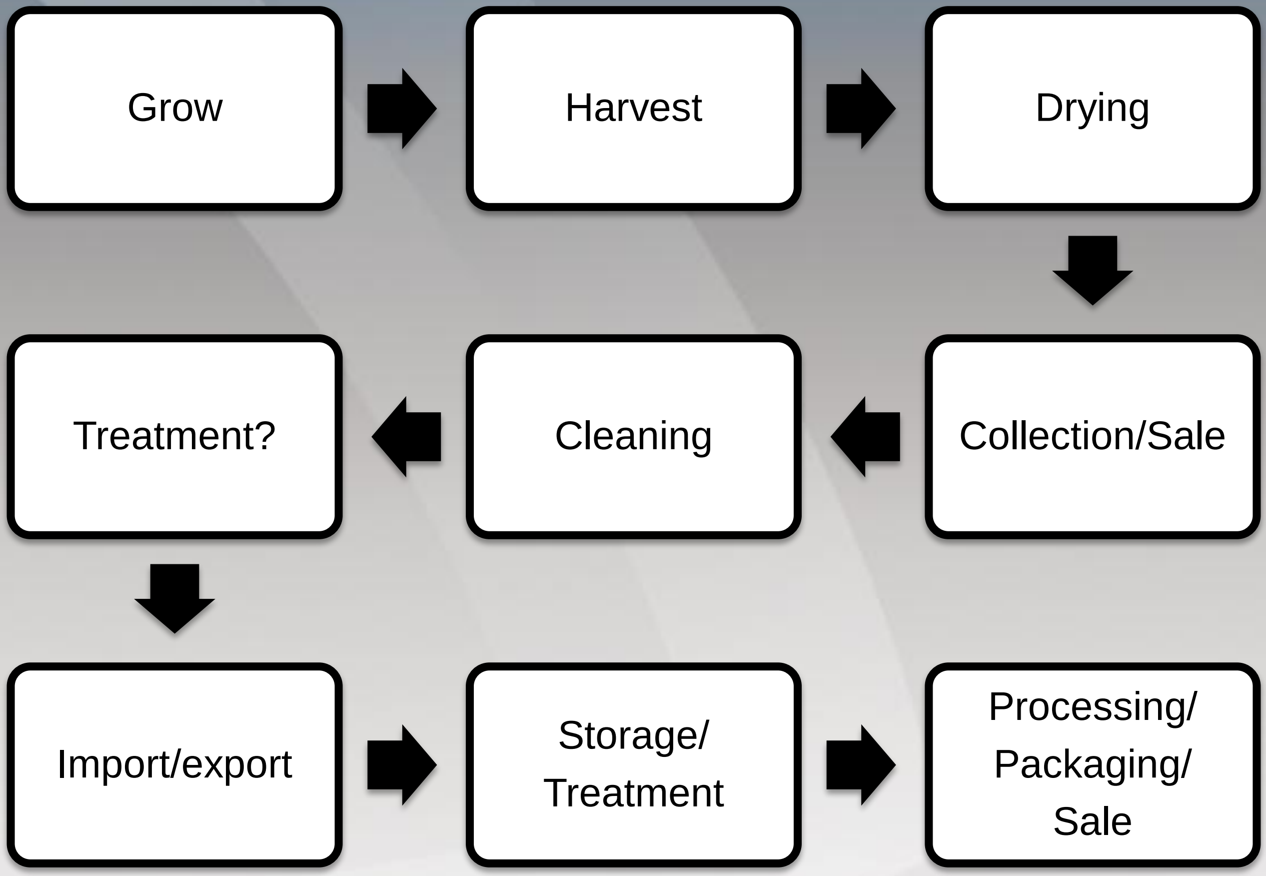
CFSAN

Spices: production/source



Abbreviated diagram: ASTA 2011

Spices: production/source

***Piper nigrum**:*
Grows as a vine,
trellising is required or supports,
typically lives 20 years

Black pepper:
Mature green(unripe fruit), typically boiled (blanched), sundried

White pepper:
Mature ripe (red) fruit, fermented to remove fleshy coat, washed, sundried

Green pepper:
Pickled mature green fruit

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Spices: production/source

“Preservation of micro-organisms by desiccation has been the preferred method for long term storage of cultures for decades.”

Morgan, C. A., N. Herman, P. A. White, and G. Vesey. 2006. Preservation of micro-organisms by drying; A review. *Journal of Microbiological Methods*. 66:183-193.

Factors affecting survival:
Growth stage
Protective agents
Growth media

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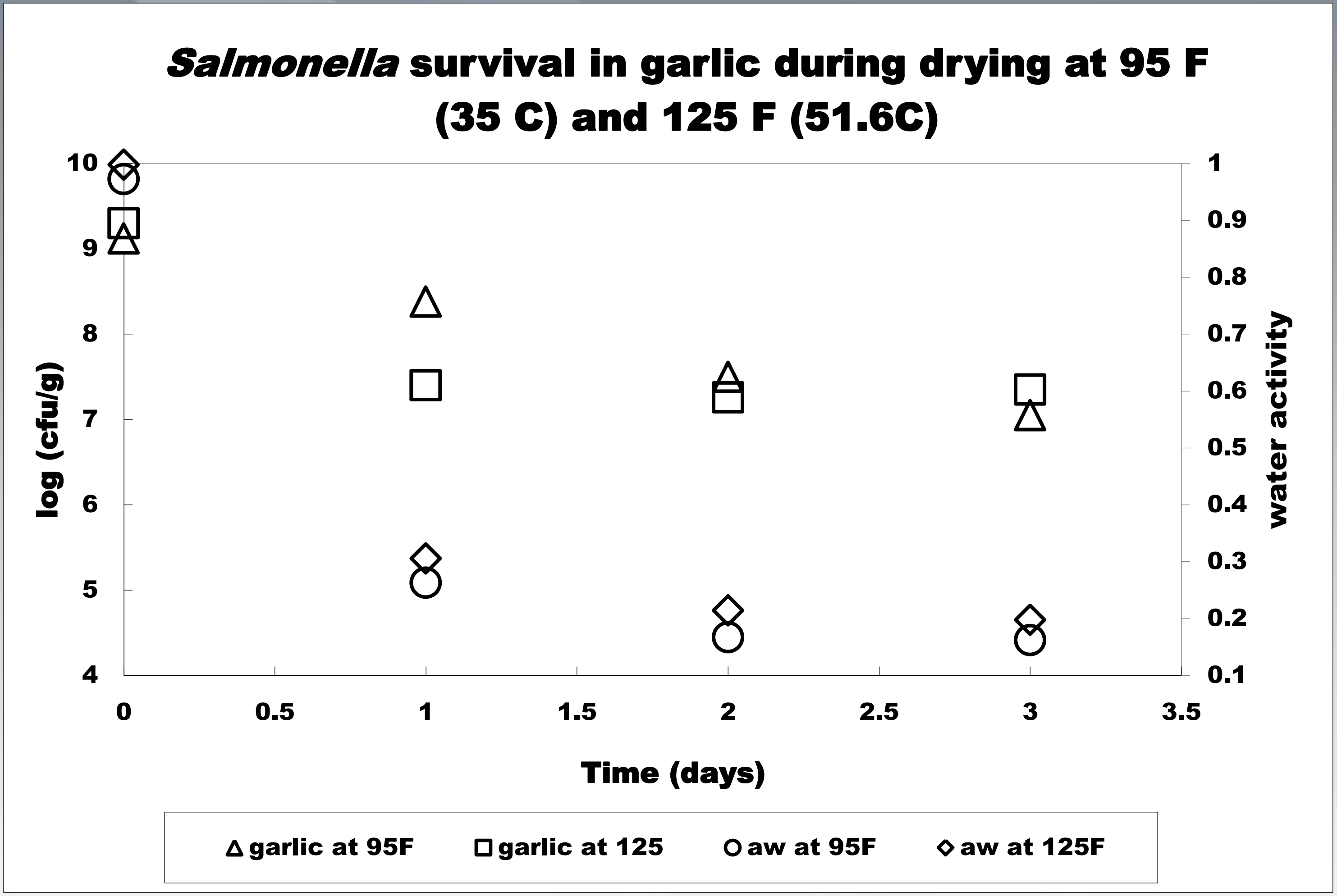
Spices: production/source



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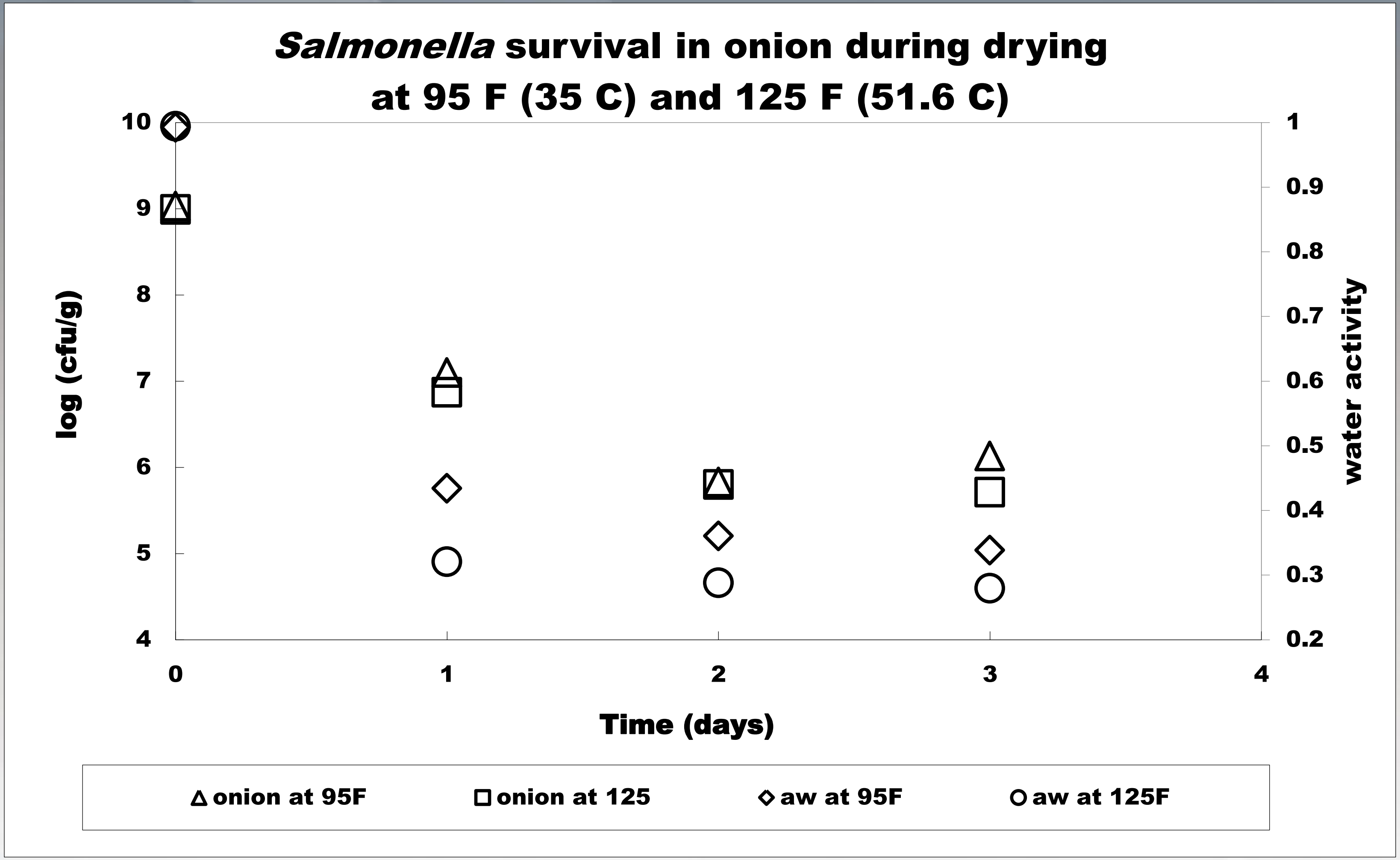
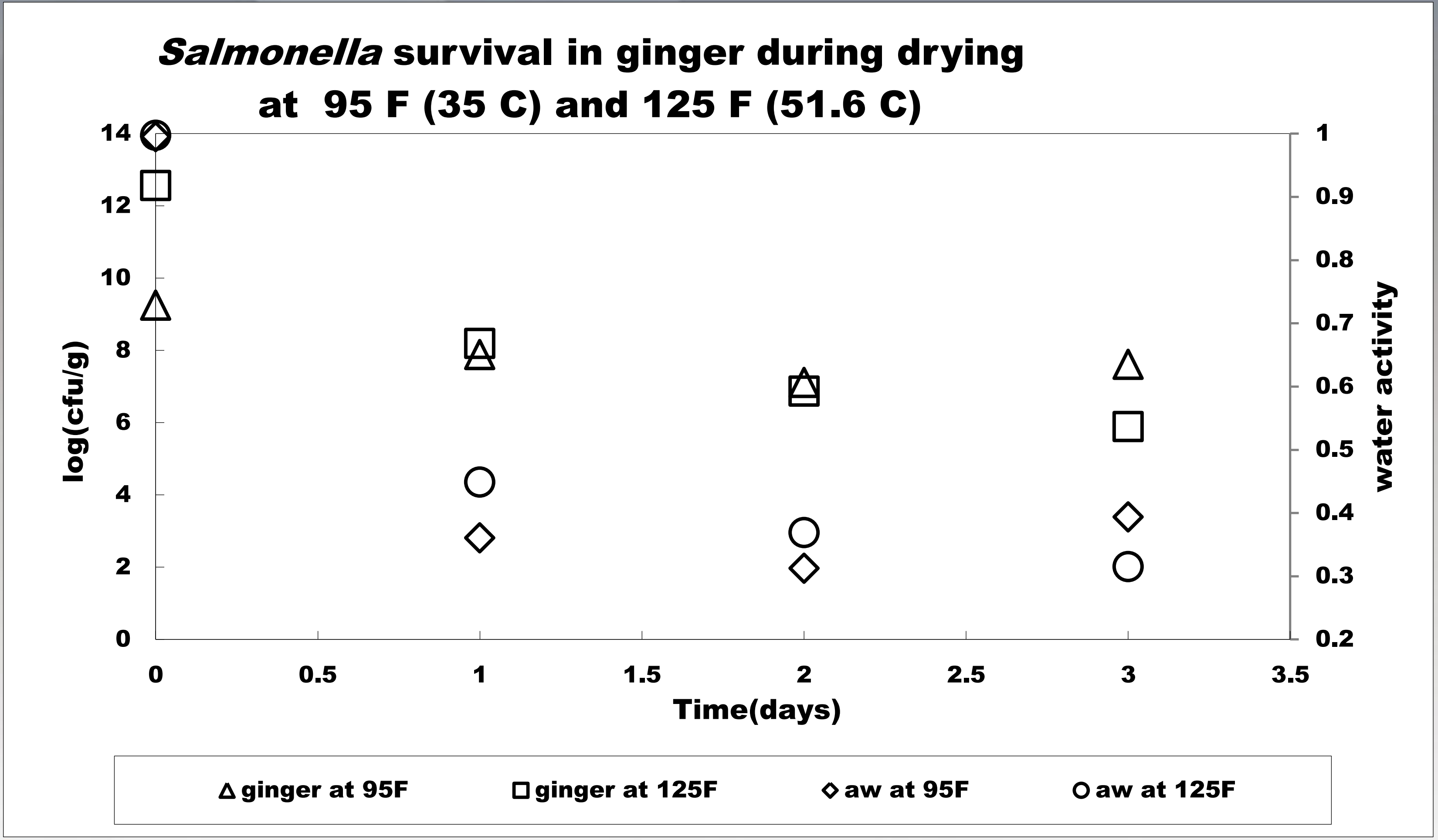
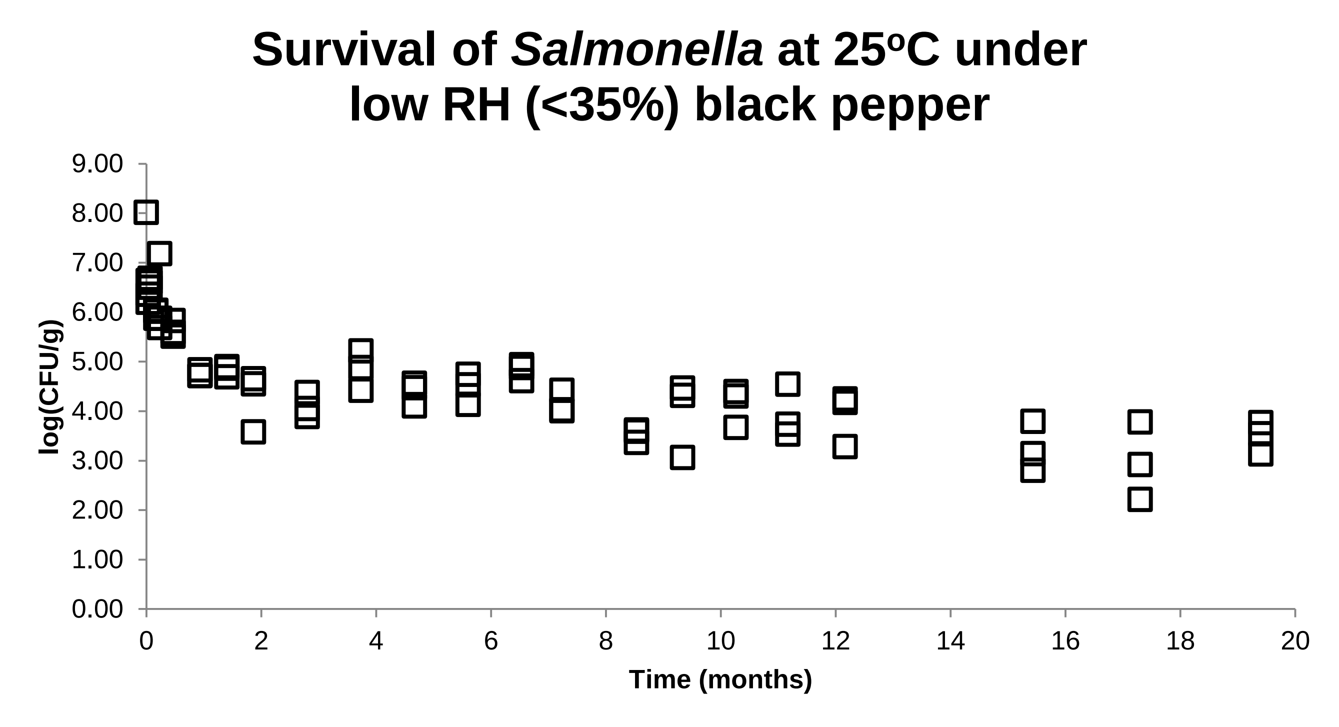
Spices: production/source



Spices: production/source



Salmonella survival in dried spices



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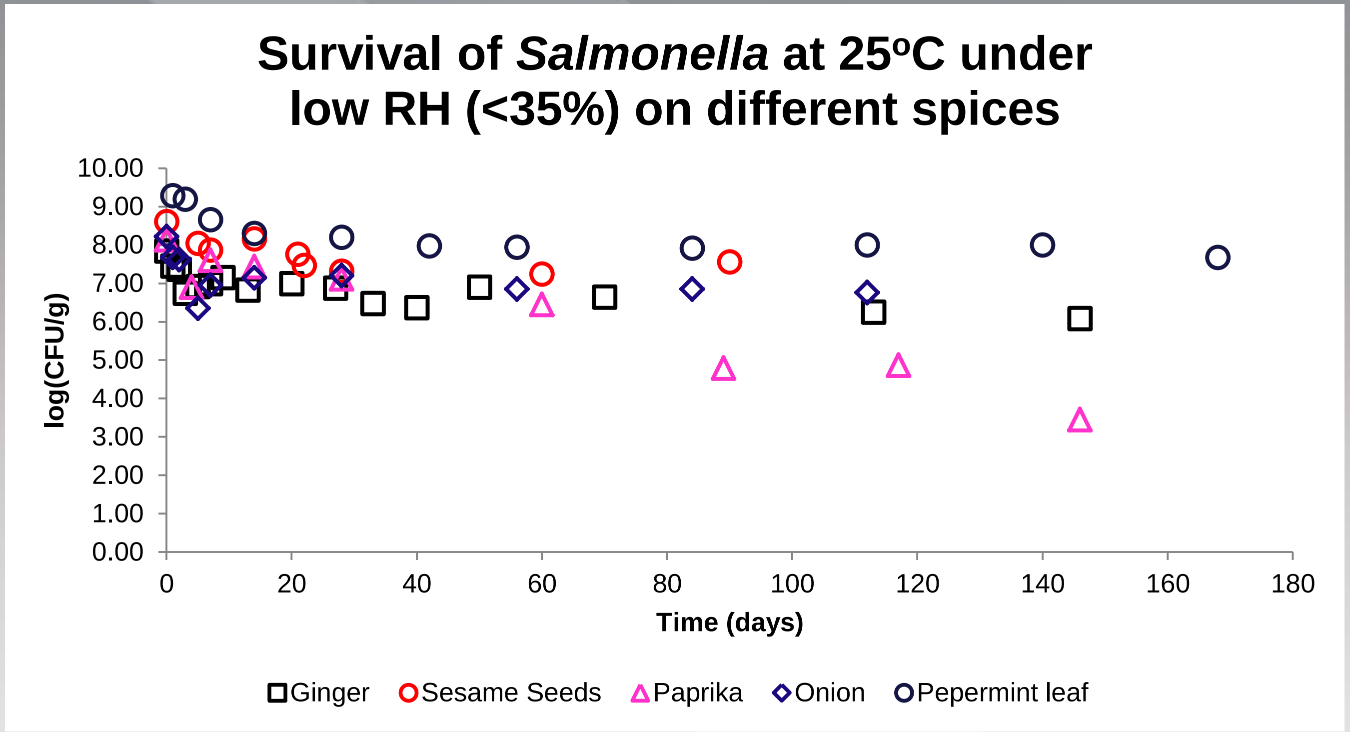
Salmonella survival in dried spices



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Salmonella survival in dried spices

Antimicrobials in spices

- Essential oils:can contain 20–80
different constituents at different concentrations
- Terpenes
- Terpenoids

Used as “natural” food preservatives

Can interfere with detection

i.e. Clove, oregano must be diluted to detect pathogens

Salmonella survival in dried spices

Spice	Dry-Transfer (10.33 ± 0.04 log CFU/g)[1]	Aqueous Suspension (11.05 ± 0.06 log CFU/mL)[2]
Clove	9.00 ± 0.37[A]	3.63 ± 0.64[C]
Oregano	9.16 ± 0.25[A]	7.47 ± 0.26[B]
Ginger	9.10 ± 0.29[A]	8.55 ± 0.46[AB]
Black Pepper	8.87 ± 0.39[A]	8.54 ± 0.25[AB]

Hildebrandt, et al. 2017. *Journal of Food Protection*. 80:338-344.

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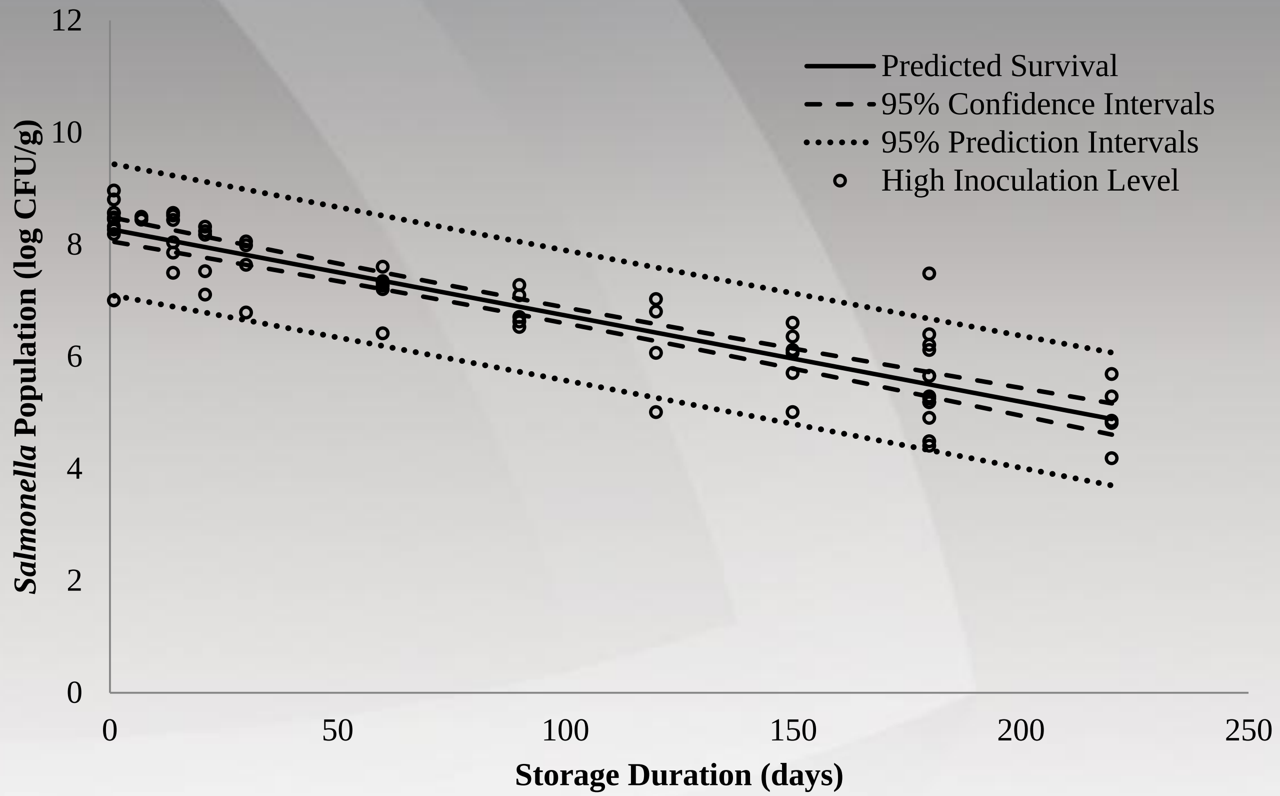
Survival of *Salmonella* in dried clove

Survival of *Salmonella* inoculated into clove powder during storage at 25°C



Hildebrandt, et al. 2017. *Journal of Food Protection*. 80:338-344

Prevention and Remediation

Incorporate GAPs and GMPs

It's Not just for Fresh Produce!!

Prevention and Remediation

Prior and adjacent land use,
Drainage and soil properties,
Fertilizer use, soil fertility
Pest and disease control
Water quality and use practices
Equipment and container sanitation,
Worker hygiene and sanitary facilities
Harvest implement and surface sanitation,
Effects of domesticated animal and wildlife
Postharvest water quality and use practices,
Postharvest handling, transportation and distribution,
Documentation and recordkeeping.

2003 Production Practices as Risk Factors in Microbial Food Safety of Fresh and Fresh-Cut Produce. Vol. 2 (Supplement), COMPREHENSIVE REVIEWS IN FOOD SCIENCE AND FOOD SAFETY T. Suslow et al.

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What's next?

Drying is not a pathogen reduction step.

Pathogens on spices at harvest prior to drying will likely be present after drying. Additional processing or "kill" steps are needed.

Diarrheagenic *E. coli* pathotypes (included enterotoxigenic *E. coli* and Shiga toxin-producing *E. coli*): 36% of serrano pepper, and 14% of Jalapeno peppers.

Cerna-Cortes, J. F., C. A. Gómez-Aldapa, E. Rangel-Vargas, M. del Refugio Torres-Vitela, A. Villarruel-López, and J. Castro- Rosas. 2012. Presence of some indicator bacteria and diarrheagenic *E. coli* pathotypes on jalapeño and serrano peppers from popular markets in Pachuca City, Mexico. *Food Microbiology*. 32:444-447.

Low Water Activity Foods Safety Series: Part 3 of 4 Microbiological Safety of Dried Spices

DECONTAMINATION PROCEDURES AND DETECTION METHODS

MAY 12, 2021

ALEX BRANDT, PH.D., CHIEF SCIENCE OFFICER

FOOD SAFETY NET SERVICES, LTD.

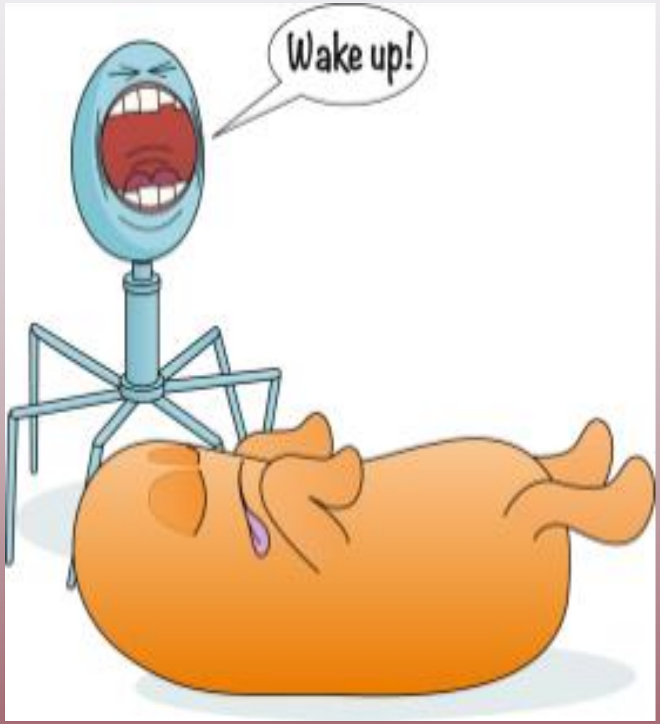
OVERVIEW

- Decontamination Methods for Spices
- Method Validation Considerations for Spices

DECONTAMINATION

- Susanne:

  "Drying is not a decontamination step."
- Drying will actually preserve pathogens for long-term survival in a dormant state.
- Need other post-drying decontamination procedures to reduce the viability of pathogens in spices.
- How do we reduce them?
- Primary choices for decontamination include:
  - Steam Treatment
  - Ethylene Oxide Treatment (EtO)
  - Irradiation



STEAM TREATMENT

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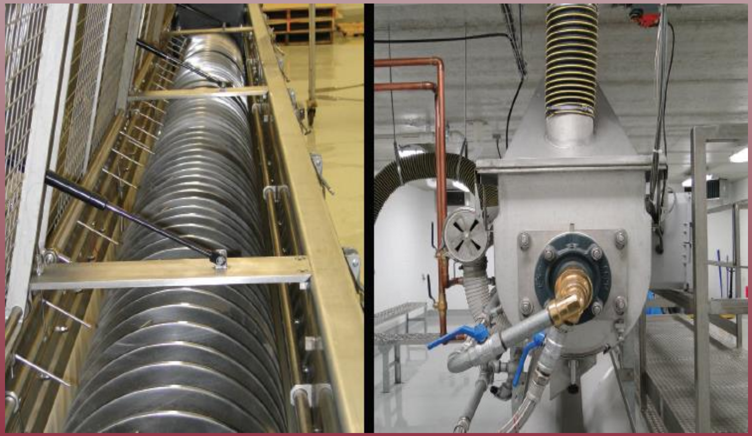
- Greater consumer acceptance than other methods (EtO and Irradiation).
- Both wet and dry steam applications.
- Temperatures and times vary:
  - 75-85°C, 100-120°C, 150-160°C (Superheated Steam)
  - Times of 20-40 s on low end, up to 10 min.
- Decrease in quality from high temperatures used with longer processing times.
- Can cause volatile oil reductions and discoloration in some spices, such as paprika/dried red pepper.



STEAM TREATMENT

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- Newkirk, et al. 2016 – evaluated vacuum-assisted steam to inactivate *Salmonella enterica* on whole black peppercorns and cumin seeds at one pressure (16.9 PSIA) and two commonly used reference temperatures (165°F and 180°F). Data from 165°F are shown below. Red lines represent 5.0 log CFU/g reduction levels.

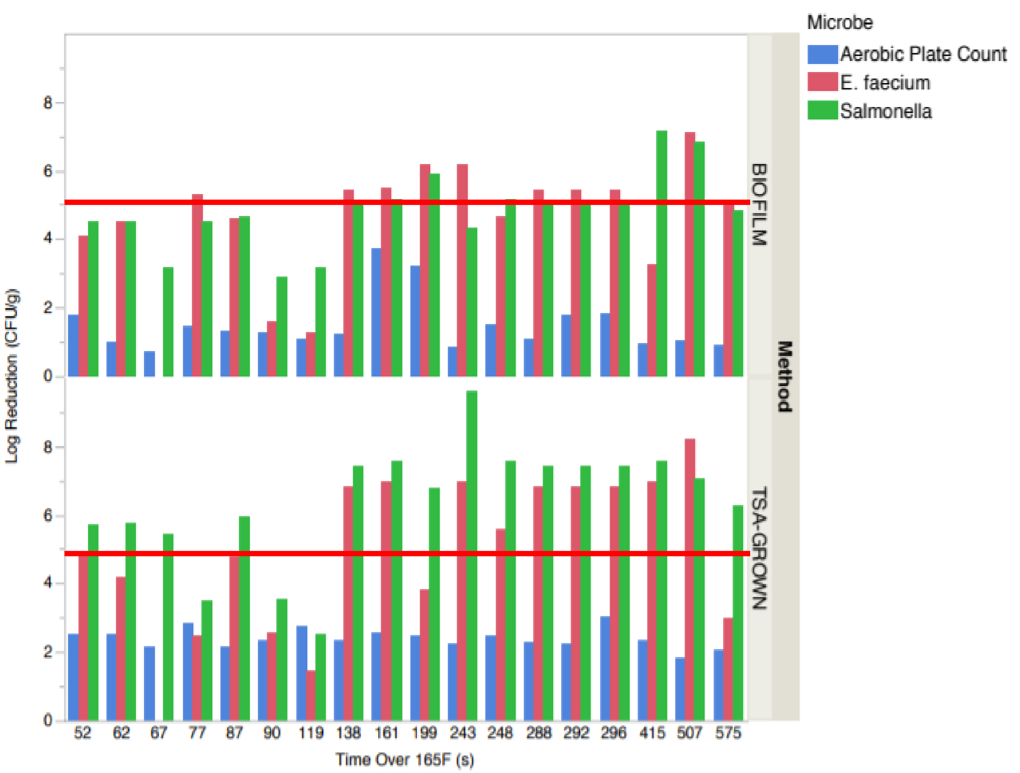

Figure 5. *Log reduction (CFU/g) of Salmonella enterica and Enterococcus faecium inoculated using various methods onto whole black peppercorns processed using a lab-scale steam apparatus associated with varying exposures to temperatures above 165°F. Each bar represents one run.*

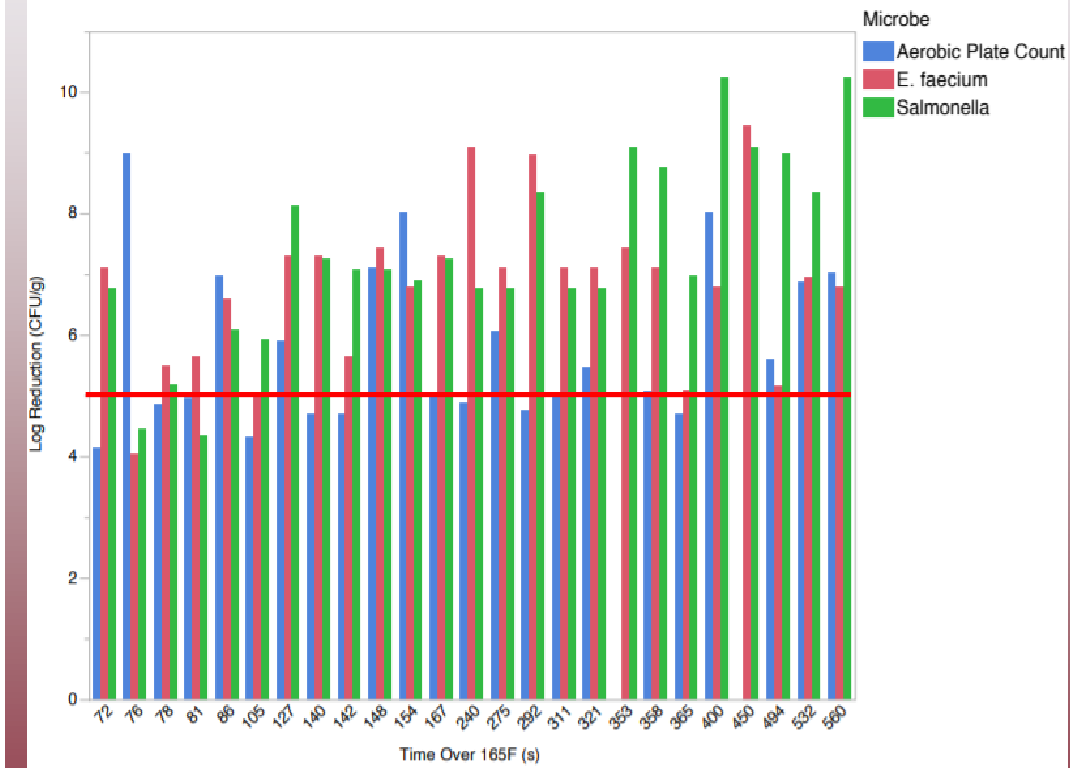

Figure 9. *Log reduction (CFU/g) of Salmonella enterica and Enterococcus faecium inoculated cumin seeds processed using a lab-scale steam apparatus associated with varying exposures to temperatures above 165°F*. Each bar represents one run.

STEAM TREATMENT

- Shah, et al. 2017 – also evaluated vacuum-assisted steam to inactivate *Salmonella* Enteritidis PT30 on whole black peppercorns at 75-105°C and achieved 5.0 log CFU/g reductions in as little as 30 seconds. Red lines represent 5.0 log CFU/g reduction levels.

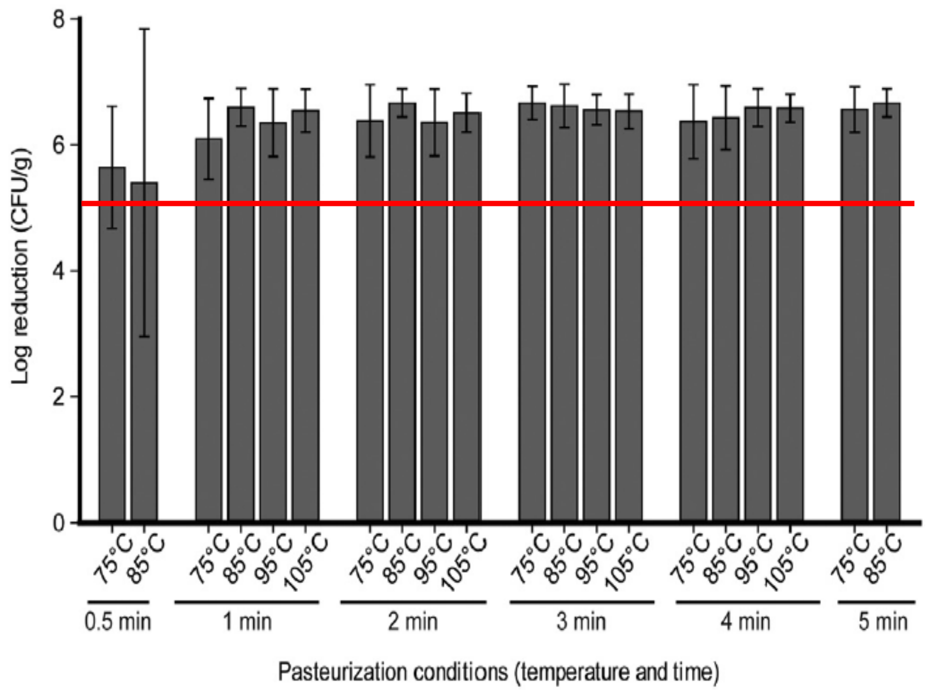

Fig. 5. Average log reduction in CFU/g after vacuum steam pasteurization of whole black peppercorns for *Salmonella* PT 30 at pasteurization temperatures of 75, 85, 95 and 105 °C. Log reductions are average of three independent replicates and three technical replicates for each strain. Error bars denote standard deviations.

ETHYLENE OXIDE (EtO)

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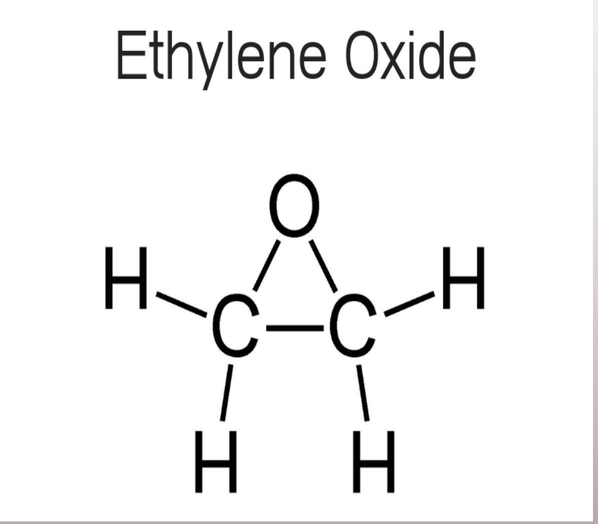
- Colorless gas with high degree of penetration.
- EPA-registered antimicrobial pesticide used for sterilization of medical devices and spices. Residue limits are established for food products.
- Mode of Action: Causes alkylation of organic compounds such as enzymes, proteins, and nucleic acids, essentially inactivating them.
- Can reduce bacterial loads but there is concern about loss of color and flavor due to volatile oil loss.
- EPA is currently reviewing EtO use due to health concerns; not allowed in Europe.
- Between 40 and 85% of spices in US are treated with EtO according to ASTA.



ETHYLENE OXIDE (EtO)

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- Chen, et al. 2021 investigated the effects of relative humidity (30, 40, and 50%), temperature (46, 53, and 60°C), and exposure time for EtO inactivation of *Salmonella* spp. on cumin seeds
- Also evaluated *Enterococcus faecium* NRRL B2354 as a suitable surrogate for *Salmonella* inactivation on cumin seeds.
- Twenty minutes of EtO treatment at 50% relative humidity achieved ~5.0 log reductions of both bacteria at all three temperatures.
- Weibull modeling indicated that sensitive cells were inactivated first, and hardy ones survived at low relative humidity treatment conditions.
- Log reductions of both bacteria on cumin seeds increased with the increasing relative humidity and temperature for EtO treatment. Thus, relative humidity is a critical factor for successful EtO inactivation treatment and it must be higher than 40% to implement a successful and efficient EtO decontamination of cumin seeds.
- *E. faecium* consistently showed lower log reductions than those of *Salmonella* under all EtO treatment conditions investigated in this study, demonstrating that *E. faecium* is a suitable surrogate for *Salmonella* for evaluating EtO treatment on cumin seeds.

ETHYLENE OXIDE (EtO)

- Carver, et al. 2016 used 20% EtO/80% CO_2 at 130°F for 300-350 min to treat whole black peppercorns and cumin seeds inoculated with *Salmonella enterica*. Resulted in a mean 6.6 ± 0.6 log CFU/g reduction for peppercorns. However, a 5.0 log CFU/g reduction was not achieved for each sample. Likewise, a 5.0 log CFU/g reduction did not occur for cumin seeds for every sample.

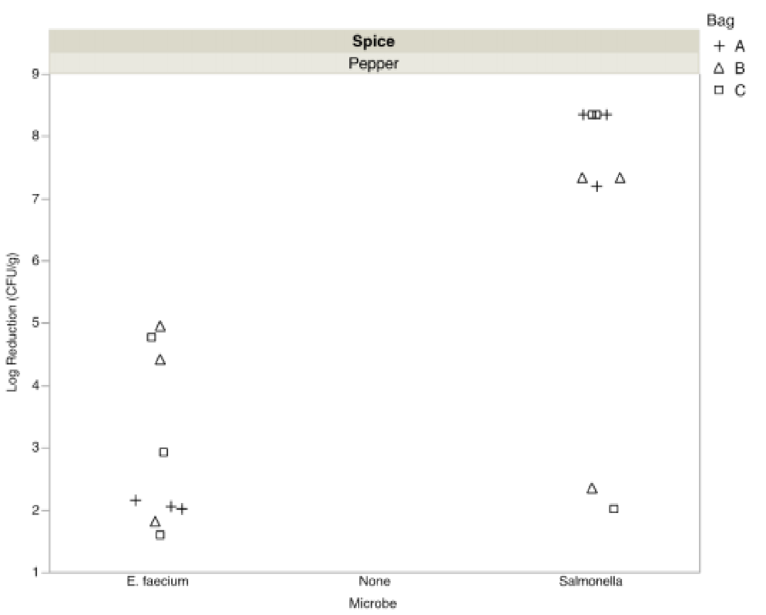


Figure 1. Log reduction of *Salmonella enterica* and *Enterococcus faecium* on whole black peppercorns after treatment with ethylene oxide.

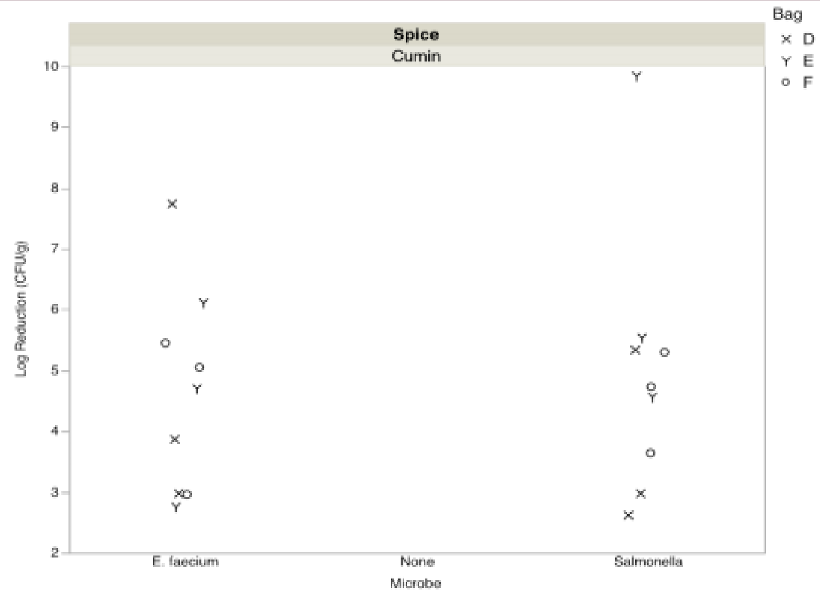
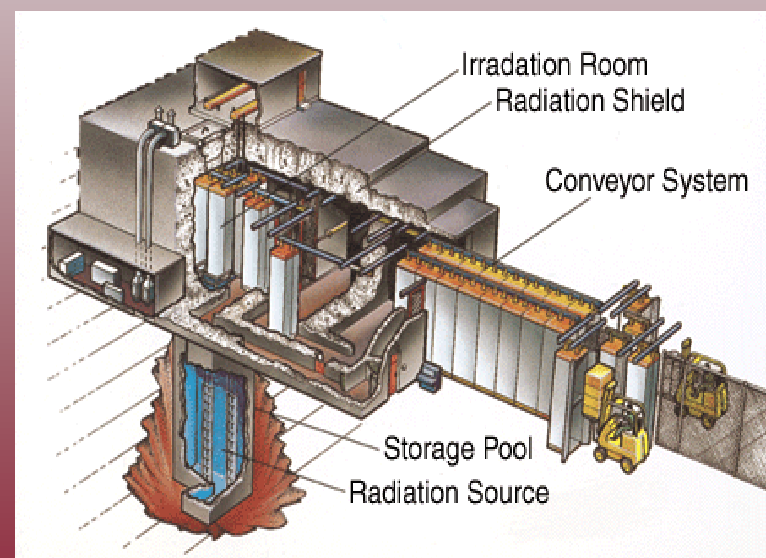


Figure 2. Log reduction of *Salmonella enterica* and *Enterococcus faecium* on cumin seeds after treatment with EtO fumigation.

IRRADIATION

- High doses of ionizing radiation used to generate microbicidal compounds.
- Energy-efficient – use gamma, electron-beam, and X-ray radiation. Dose: ≥ 10 kGy
- Mode of action: high energy particles collide with and break chemical bonds to create short-lived radicals, which cause further chemical changes by bonding with and or stripping particles from nearby molecules. When DNA or RNA are damaged, reproduction becomes hindered, which halts microbial growth.
- Consumer acceptance has been low for any kinds of products that have been subjected to irradiation. Requires special labeling (Radura).



IRRADIATION

- Song, 2014 et al. evaluated efficacy of gamma irradiation to inactivate *Escherichia coli* O157:H7 and *Salmonella* Typhimurium in black and red pepper.
- Black and red pepper were inoculated and subjected to gamma irradiation up to 5 kGy, and color change was evaluated after treatment.
- 5 kGy decreased *E. coli* O157:H7 and *Salmonella* populations by > 4.4 to > 5.2 log CFU/g in black pepper without causing color change.
- 5 kGy yielded reductions of 3.8 to > 5.2 log CFU/g for *E. coli* O157:H7 and *Salmonella* in red pepper. Minimal color changes were observed.
- *Salmonella* was more resistant to gamma irradiation than was *E. coli* O157:H7.

IRRADIATION

- D'Oca, et al. 2021 used ^{60}Co gamma irradiation to treat black pepper and sesame seeds that were contaminated with *Salmonella* Montevideo. Treated with 1, 3, and 5 kGy. Observed > 5.0 log CFU/g reductions with a 5 kGy treatment.

Table 4. Reduction of *S. Montevideo* in black pepper and sesame seeds after irradiation treatment (means of three replications ± standard deviation).

Dose (kGy)	Reduction of *Salmonella* spp. (log CFU /g)	
	Black pepper	Sesame seeds
1	1.35±0.04	1.96±0.13
3	3.22±0.16	4.17±0.11
5	6.02±0.04	5.53±0.19

OTHER METHODS

- Other spice decontamination methods have also been investigated:
- Near Infrared Radiant Heating
  - Fast and effective thermal process.
  - IR radiation is part of the electromagnetic spectrum and has advantages over convection and conduction heating, as it heats the product directly without being influenced by air around the powdered spices
  - Can be used to improve the sealing of moisture, flavor, and aroma compounds, leading to better sensory.
- UV Radiation
  - Nonthermal method approved for use as an antimicrobial treatment of food surfaces.
  - Most effective in combination with other techniques to damage microbial DNA and decrease the overall number of bacterial cells.

METHOD VALIDATION CONSIDERATIONS FOR SPICES

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- Spices are one of the most challenging food groups for method development, especially rapid methods.
- Antimicrobial compounds like eugenol (clove), cinnamaldehyde (cinnamon), carvacrol/thymol (oregano), piperine (black pepper), allicin (garlic), et al. inhibit pathogen growth in enrichments and interfere with assay chemistry.
- Also physical properties of the spices themselves (i.e. small particles) can interfere with the detection assay chemistry.

SPICE GROUPINGS

- Best Practices for Microbiological Methodology from FDA:

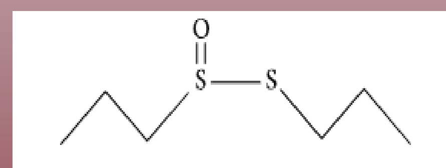
Category 8 – Spices*

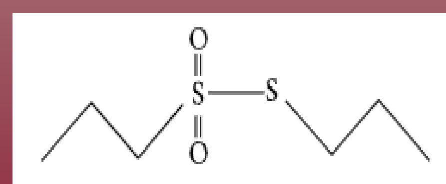
Class	Group
1	Black Pepper, White Pepper, Caraway Anise, Celery, Cumin, Dill, Fennel, Nutmeg, Coriander, Ginger, Paprika
2	Onion, Garlic
3	Oregano, Cinnamon, Allspice
4	Thyme, Marjoram, Basil, Sage, Rosemary
5	Red Pepper, Chili Pepper
6	Cloves

*Spices are a particularly troubling category as many contain uncharacterized naturally occurring compounds that can interfere with detection assays. Although this table can serve as a guideline, the user is strongly encouraged to consult the literature and perform preliminary experiments on each spice to demonstrate that the method performs as expected with new matrices before routinely using the method on those matrices.

SPECIAL MODIFICATIONS

- FDA BAM Chapter 5: *Salmonella* Section C Part 10 Spices
  - Onion Flakes, Onion Powder, Garlic Flakes
    - Contain propyl disulfides that react with thiol groups of various enzymes (such as the acetyl-CoA system) that are involved in lipid synthesis and inhibit RNA polymerase and RNA synthesis
    - Add 0.5% K_2SO_3 into the TSB enrichment
    - K_2SO_3 will neutralize propyl disulfides in onion and garlic





SPECIAL MODIFICATIONS

- FDA BAM Chapter 5: *Salmonella* Section C Part 10 Spices
  - Allspice, Cinnamon, Cloves, Oregano
    - No means to neutralize toxicity for enrichments
    - Powder forms of these spices must be diluted extensively
    - Must dilute allspice, cinnamon, and oregano to a 1:100 sample:broth ratio
    - Must dilute cloves at a 1:1000 sample:broth ratio




SPECIAL MODIFICATIONS

- FDA BAM Chapter 5: *Salmonella* Section C Part 10 Spices
  - Allspice, Cinnamon, Cloves, Oregano
    - Whole chunks, pieces, and leaves get 25 g added to 225 ml TSB
    - Shake vigorously for 60 s and then transfer the rinsate to a fresh bag
    - Cannot allow them to soak! Must transfer immediately!
    - Continue enrichment with rinsate




VALIDATED RAPID METHODS

- bioMérieux VIDAS®
  - SLM Easy and SPT Assays: Ground Black Pepper
- bioMérieux GENE-UP®
  - SLM 2: Garlic Powder
- Hygiena BAX®
  - Standard *Salmonella:* Black Pepper
  - RT *Salmonella*: White Pepper, Whole Black Pepper, Dried Parsley
- Millipore-Sigma Assurance GDS®
  - *Salmonella* Tq: Curry Powder, Chili Powder, Cumin Powder
- 3M™ MDA
  - *Salmonella* 2: Black Pepper

FSNS® Food Safety Net Services

VALIDATED RAPID METHODS

FSNS®
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- Hygiena BAX® RT *Salmonella* Special Modifications:
  - White Pepper:
    - 25 g into 225 ml, Incubate, then Regrow 10 µl in 500 µl BHI
  - Whole Black Pepper:
    - 25 g into 75 ml, Incubate, then dilute 80 µl in 2 ml PBS
  - Dried Parsley:
    - 25 g into 225 ml, Incubate, then dilute 80 µl in 2 ml PBS

RAPID METHOD CHALLENGES

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- Customer requested 375 g Ground Cloves testing with Hygiena BAX® RT *Salmonella*
- Verification Trial 1:
  - Liquid inoculum
  - 1:100 dilution; 1:1,000 dilution for 375 g would require 375 L of media! Not feasible.
  - All dead
- Verification Trial 2:
  - Dry inoculum pellet mixed with milk powder
  - 1:10 dilution with 25 g; collected rinsate through a filter bag
  - All dead

RAPID METHOD CHALLENGES

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- Verification Trial 3:
    - Dry inoculum pellet mixed with milk powder
    - 1:10 dilution with 25 g; collected rinsate through a filter bag
    - Added 9 ml of 10% Polyvinyl pyrrolidone (PVP) to 225 ml filtrate
    - All dead
- Final Decision: Rapid method could not be verified to test 375 g samples, even when breaking down to 25 g, diluting, rinsing, and adding PVP to neutralize eugenol.
- Tested as 3.75 g sample with normal 1:1,000 dilution conditions according to FDA BAM Chapter 5

TAKE-HOME MESSAGES

- Spice decontamination methods include steam, EtO, irradiation, near infrared radiant heating, and UV radiation
- Can achieve 5.0 log CFU/g reductions with correct parameters
- Spices are a challenge for pathogen detection methods
- Modifications have to be made for rapid and cultural methods
- Method modifications are not guaranteed to work

THANK YOU!

QUESTIONS?

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Questions?

Questions should be submitted to the presenters via the **Questions section** at the right of the screen.

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