Guidance on Science-Based Groupings to Optimize Validation of Spice Process Controls

Date of Last Revision: 10 May 2022

Revision No. 2

Reason for Revision: Updated Information

Table of Contents

Introduction 3

Considerations for Validation 4

Considerations for Process Validations Based on Groupings 5

1. Process-Related Considerations 5

2. Intrinsic Physical Factors 6

Examples of Validations of Spice Groupings 10

Example 1 10

Example 2 11

Example 3 11

Example 4 11

Summary of Examples 12

Conclusions 14

Cited References 15

Appendix 1. Additional Resources 19

Appendix 2. Properties of process - Guidance for determining conditions for a validation of an antimicrobial process for spices. 21

Appendix 3. Outline of a hypothetical validation experiment. (Following the general guidance of ISO 20976-1). 22

Introduction

Purpose: The document provides a scientific and technical justification for selecting a subset of spices for validation, with the understanding that once the subset is validated, the results are applicable to the entire predefined group of spices.

Scope: This document provides a framework for validating a lethality process to achieve a specific level of pathogen reduction in groupings of spices. It addresses the three most commonly used interventions, steam, gas, and irradiation, although the principles outlined in this document have the potential be applied to alternative technologies as they are developed. While this document does not provide a comprehensive overview of validation study considerations, ASTA has developed several additional resources to support members including a White Paper and Webinar Series on Validation of Microbial Reduction Processes for Spices available at www.astaspice.org.

Background: The U.S. Food and Drug Administration (FDA) regulations on Preventive Controls for Human Foods (PCHF) establish legal requirements related to mitigating microbiological contamination of spices, including implementing process controls. The spice industry has historically relied on several different microbial reduction processes, including heat (steam), gas, and irradiation. The PCHF regulations also require validation for such process controls. Validation must include obtaining and evaluating scientific and technical evidence to determine that the process controls, when properly implemented, will effectively control the hazard. For the hazard of non-typhoidal *Salmonella enterica*, there is a general expectation that the process control will achieve a minimum 5 $\log_{10}$ reduction in the population of a pathogen.

Spice companies typically process a wide variety of spice and herb products, which are sold both individually and as blends. Given the wide array of spice ingredients being processed in the same facility, it is resource prohibitive and often redundant to run a full validation study on each product. When considering opportunities to group spice and herb products for purposes of validation, it is important to recognize that process efficacy is both product and process dependent. Accordingly, process-related and inherent physical parameters must be evaluated to ensure that the representative commodity in a grouped validation study represents the "worst-case" scenario.

Considerations for Validation

It is scientifically appropriate to use a challenge study conducted on one (or two) product(s) to validate a group of similar products, so long as there is an evidence-based justification that the results should apply to a broader group. It is FDA's policy that challenge studies on one product may sometimes be applicable to other products (78 Fed. Reg. 3636, 3753-54 (Jan. 16, 2013). FDA has stated that the more similar the composition of the products, the more likely that a study on one product will apply to a grouping of products. However, a challenge study may not be applicable when significant differences in intrinsic properties between products exist. Consideration should be given to intrinsic properties of the like products that may impact the survivability of the target pathogen for a given process. The physical method of inoculation of the spice(s) with an appropriate surrogate for validation is an important consideration, as the inoculation method may affect the outcome (Bowman et al., 2015; Hildebrandt et al., 2017)

Key intrinsic properties to consider for validating a process within a specific grouping of spices may include particle size, density, physical shape, water activity, type of the plant, product form, and inherent antimicrobial properties. Other considerations may include process stage, seasonal variation, thermal properties, pH, salt, fat, carbohydrate, and protein content. Process-specific considerations may include process technology, mode of inactivation, percent of capacity used, cold-spot variability or bulk packaging used during processing.

The physical parameters of the spice will affect the process and the response of the bacteria to the process. As an example, the thermal resistance of non-typhoidal *Salmonella enterica* and *Enterococcus faecium* increases as the water activity decreases in both food and nonfood matrices (Archer, et al., 1998; Liu et al., 2018; Park et al., 2021). In addition, the density of the product affects the time required to obtain a minimum radiation absorbed dose or heat transfer at the center of the product and can affect the ability of a sterilant gas to penetrate the product.

The representative spice product(s) selected to validate a group should represent the "worst-case" scenario with regards to these parameters, meaning the product that would be most likely to promote survivability of the pathogen. Choosing the spice with the characteristics that would make it most difficult to control *Salmonella* in the group ensures that the rest of the spices in the group should also be controlled, at least to the extent of the product tested.

A common approach to grouping spices when validating microbial interventions is to group the spices by the part of the plant from which the spice is derived, because spices derived from the same plant part are likely to have similar characteristics, such as density. However, a variety of approaches may be scientifically valid. It is the consensus opinion of an ad hoc committee formed by the American Spice Trade Association, made up of industry experts, that the characteristics of spices that may affect validation include their physical properties, such as density, water activity, and particle size distribution. It is important to recognize that this is still an emerging area, and there is not a yet a strong scientific body of evidence regarding the

impact of physical properties of spices on survivability of *Salmonella* in process controls. Companies may choose to develop groupings on a variety of relevant similarities of intrinsic properties (e.g., density, fat composition, known antimicrobial properties), during categorization or selection of the representative product for a group. It is also important to consider the variability of physical properties from batch to batch within each spice, and to consider how that might impact the process at the extreme of that variability.

Considerations for Process Validations Based on Groupings

1. Process-Related Considerations

a) **Process Technology**: The primary initial consideration should be the type of process to be validated. The parameters that will most likely impact the outcome of the process are inherently related to the mode of action of the process. The common processes used to decontaminate spices, including but not limited to steam, gas, and irradiation, all inactivate bacteria by different biological mechanisms. As such, the critical parameters will vary between processes. Examples of some of the critical parameters for specific processes are listed below:

i. **Steam:** The density and water activity of the product affect the heat transfer into the spice and will need to be determined for each spice. Additionally, steam temperature and pressure (atmospheric or higher), exposure time, vacuum (if used), come-up time or prewarming, flow rate (if continuous process), packaging (if batch processing) are important processing parameters to take into account, along with the heat transfer of the specific product.

ii. **Gas**: density of the spice, gas concentration, gas pressure, properties which affect gas penetration, relative humidity, temperature, exposure time

iii. **Irradiation**: density of the spice, minimum absorbed dose (dose mapping)

Treatment process parameters play a key role in determining the worst-case scenario. For example, ethylene oxide (ETO) shows greater reduction of foodborne pathogens when the relative humidity during the treatment is increased (Wei et al., 2021). If the relative humidity range for an ETO process is between 30-50%, then a process validation should be conducted using the lower end, thus airing on the side of caution, and using a worst-case processing scenario. More details on potential sources of process validation variability that need to be considered, such as those related to the equipment (e.g. batch versus continuous process, size of load, depth of product, etc.) are available in ASTA's White Paper and Webinar on Validation of Microbial Reduction Processes for Spices.

b) **Packaging**: The nature of the packaging of the spices may impact the ability of an intervention to be effective. As an example, if a spice will be processed with gas, the packaging must be permeable to the gas. The size and arrangement of the packages during the process may also impact the effectiveness of the intervention. These considerations are normally determined during the performance qualification phase of the process (e.g., dose mapping for irradiation).

2. Intrinsic Physical Factors

a) **Part of the plant**: The part of the plant may be an initial factor to consider when determining classification groups. Materials grown on the same part of the plant typically have similar physical characteristics, especially when compared to materials from other parts of plants. For example, herbs have more surface area and lower density compared to berries. Accordingly, a validation process developed for cilantro would be more likely to translate to other herbs compared to black pepper.

b) **Product form**: The second consideration for a product classification system is the product form. During processing, the form of a spice or herb is often changed through cutting or milling. Ground product will have higher surface area and higher density compared to its whole form and could arguably be considered a completely different food product. Thus, when designing validation experiments there may be different considerations for a ground product than for its whole form. In addition, differences in the physical size and shape of the whole product may impact the results (Saunders et al., 2018)

c) **Water activity**: The amount of water or moisture in a product that is not chemically bound with other components of the foods, such as salt, is commonly measured as water activity (a_w) and impacts microbial survival and resistance to intervention processes. In general, the lower the water activity, the more resistant bacteria are to thermal resistance (Syamaladevi et al., 2016; Liu et al., 2018). Fumigation processes, such as ethylene oxide (ETO), are presumably also significantly impacted by the water activity, since reduced relative humidity (and subsequent reduction of the water activity) reduces the efficacy of the treatment (Wei et al., 2021). Meanwhile, the situation for irradiation is less clear. Studies have shown a non-linear relationship between water activity and bacterial resistance in irradiation treatments of almonds, walnuts, wheat, and dates (Steinbrunner et al., 2019; Jeong et al., 2012). In some cases, lower water activities were generally associated with decreased resistance to irradiation treatment. Because of this, the expected range of water activities of the product (Table 1) would have to be taken in to account during the performance qualification and validation of the process.

Table 1. Typical water activities of spices (Voelker et al., 2020).

Water Activity (a_w) Range	Spices
0.3 to 0.4	Garlic, onion, cumin, coriander, oregano powder and parsley leaves
0.4 to 0.5	Basil leaves, basil powder, rosemary powder, chili powder, mustard, paprika, curry powder, allspice and oregano powder
0.5 to 0.6	Black pepper, cinnamon, nutmeg, cayenne, oregano leaves, mace, turmeric and ginger
0.6 to 0.65	Cloves

d) **Density**: The density of the spice to be processed may have an impact on the effectiveness of the process. Density is an inherent property of the material. It is impacted by the material composition, the particle size, and the water activity. Some examples of bulk and particle densities are shown in Table 2. Irradiation of spices is impacted by the product density (IAEA, 2015). As the density of the spice increases, the variation within the process (the Dose Uniformity Ration (D_{UR}) or "max/min ratio") increases. The density would have to be considered during performance qualification and validation of the process.

Table 2. Examples of density of various spices (Ozturk et al, 2018)

Spice	Bulk Density (g/cm^3)	Particle Density (g/cm^3)
White Pepper	0.539	1.01
Black Pepper	0.487	1.27
Red Pepper	0.423	1.07
Paprika Powder	0.438	1.21
Curry Powder	0.401	1.15

e) **Interactions**: The two physical factors previously described (a_w and density) may interact to affect microbial survival and resistance. As an example, a spice with a higher water activity may also have a higher density. The higher water activity may make the microorganisms more sensitive to a process, but the higher density may affect the actual performance of the process.

f) **Inhibition**: Some spices have inherent properties with bacteriostatic or inhibitory effects on microorganisms (Billing and Sherman, 1998). These properties could impact a variety of aspects of validation of treatment methods, including the potential impact on surrogate organism selection, inoculation practices, and the selection of a representative product for a grouping of spices.

1. Impact on Surrogate –
   Although the issue of inhibition has been raised as a potential concern for selection of a surrogate organism in validation studies, research conducted by

the American Spice Trade Association has demonstrated that a recommended surrogate for spice validation studies, *Enterococcus faecium*, is inhibited to the same degree or less than non-typhoidal *Salmonella* in spices (ASTA, 2019). Therefore, inhibition of *E. faecium* by spices is not a significant issue for validation studies since, as a surrogate, it maintains greater resistance compared to the pathogen. Other potential surrogates for use in spice validation studies should be evaluated to determine if they are inhibited to a similar degree as *Salmonella*.

2. Inoculation Considerations –
   Antimicrobial activity in spices can pose a challenge to wet inoculation for maintaining a high inoculation level. While dry inoculation methods are commercially available, these methods are not applicable to many spice matrices (e.g., spices of large particle size). Wet inoculation is still widely used due to compatibility issues of dry inoculation methods and cost. There is strong evidence that for spices with antimicrobial activity, inoculation level can quickly decline even by several log cycles, to a level that is below 6 log CFU/g, a non-workable level to show a 5-log reduction potential (assuming the limit of detection is 1 log/CFU). Therefore, spices with inhibitory properties should be avoided when wet inoculation methods are being used.

3. Representative Product Selection –
   Questions have been raised with respect to the selection of a representative product for a spice grouping that has inhibitory properties. The concern is that if a spice with inhibitory properties is used to represent a group of spices without inhibitory properties, it could result in an overestimation of the log reduction of the treatment process. For example, assume that a highly inhibitory spice (cloves) inhibits *Salmonella* by 0.6 log, and the intervention destroys 4 logs of *Salmonella*. Therefore, the Inhibitory Spice + Intervention would result in a 4.6 log reduction. If this same intervention were to be applied to a less (or non) inhibitory spice (ginger) and the intervention were to destroy 4 logs of *Salmonella*, then the overall reduction would be Spice + Intervention = 4.0 log reduction. However, a review of the literature provides very little support for this approach with an intervention that does not change the water activity of the spice (e.g., irradiation).

   The research on the impact of antimicrobial properties of spices on treatment methods to control pathogens is summarized in Table 3 below. The majority of these studies have been conducted for irradiation and gas treatment methods. The preponderance of experimental evidence shows that inhibitory properties of spices do not impact microbial population reduction. However, there is limited evidence on steam treatment methods.

It is well known that antimicrobial compounds in water phase are highly inhibitory against microorganisms (Shelef et al 1984). The effect of antimicrobial compounds in low-moisture spices can be activated and enhanced with added water content while being treated, which is exactly the case for steam, although most of the added water is later removed by a drying process. Given the limitations in the experimental studies on the impact of inhibition for steam treatment methods, it may be prudent to select a less inhibitory spice as a representative product for the validation of a grouping in steam treatment methods.

In summary, consideration should be given to the potential impact of inhibitory properties of spices on the selection of a surrogate, the inoculation method, and the selection of a representative product for a grouping. Experimental studies have concluded that inhibitory properties of spices do not inhibit *E. Faecium* to a greater degree than *Salmonella* and it is therefore an appropriate surrogate for all spices. Additionally, inhibitory properties have the potential to interfere with wet inoculation methods. Finally, the preponderance of available scientific evidence indicates that inhibition is not a critical factor for irradiation or gas treatment methods, but spice companies should consider this factor for steam treatment methods.

Table 3. References regarding the impact of inherent spice inhibitory properties on the impact of interventions.

Irradiation	
Demonstrate no effect of inherent spice inhibition on the microbiological population reduction	Abdel-Khalek, 2008
	Alamn et al., 1992
	Eiss, 1984
	Farkas & Andrassy, 1988
	Juri et al., 1986
	Toofanian & Stegeman, 1988
	Vajdi & Pereira, 1973
	Zaman et al., 2015
Demonstrate an increase in surviving microbial population as the level of inhibition increases	Toofanian & Stegeman, 1988
Demonstrate an increase in surviving microbial population as the level of inhibition decreases	Wei et al., 2021
Gas	
Demonstrate no effect of inherent spice inhibition on the microbiological population reduction.	Chen et al., 2021. Golden et al., 2019 Toofanian & Stegeman, 1988 Wei et al., 2021
Demonstrate an increase in surviving microbial population as the level of inhibition decreases	Farkas & Andrassy, 1988

Steam	
Demonstrate no effect of inherent spice inhibition on the microbiological population reduction.	Newkirk et al., 2018

g) **Other factors**: There are a variety of other factors that could potentially impact survivability of pathogens in spices, including seasonal variation, thermal properties, pH, salt, fat, carbohydrate, and protein content. For example, while there is little research investigating the effects of the food matrix on ethylene oxide treatments, there are known interactions of fat content on steam treatments (Shah et al., 2017; Acuff et al., 2020).

3. Summary of Key Factors

In summary, several key factors may influence the survivability of *Salmonella* in a spice matrix. Table 4 summarizes the impact that key intrinsic factors have within the context of spice treatment methods. Of these factors, it appears that water activity and density may have the greatest impact on the survival of *Salmonella*.

Table 4. Impacts of Key Factors on *Salmonella* Survivability

Factor	Impact
Water Activity	As water activity increases, *Salmonella* resistance decreases in thermal treatment methods.
Density	As density increases, *Salmonella* resistance increases.
Product Form	The form of the product (ground or whole) may impact the physical parameters. A finer grind will increase *Salmonella* resistance.
Inhibition	It is well known that the effect of antimicrobial compounds in low-moisture spices can be activated and enhanced with added water, which occurs with wet inoculation and steam treatment. Therefore, if choices are available, using a spice with antimicrobial properties for validation should be avoided in these situations. However, experimental studies have shown that the efficacy of irradiation and gas treatment methods are not impacted by microbial inhibition.

Examples of Validations of Spice Groupings

The following examples illustrate these principles.

Example 1. A spice processor wishes to validate a steam treatment process for several spices: mustard (whole seed), mint (herb), basil (herb) and coriander (whole seed).

To validate the process, the processor would start by classifying the materials based on the parts of the plant and physical size. The differences between whole seeds and leafy herbs would likely not lend themselves to being grouped together, so these spices should be broken out into separate categories. Mustard and coriander are both whole seeds, allowing them to be grouped together. Mint and basil are both herbs. After determining the groupings, the processor would then select the "worst case" scenario from each grouping (e.g. product with the highest density and lowest water activity) and run the validation study on those representative products. So, each "worst case" scenario product from the herb and seed groups would be identified and validated. Once the process is validated for these representative products, it would be assumed to be validated for the other materials in the same classification group with similar physical characteristics given there are no known inhibitory characteristics of the other materials.

Example 2. A spice processor wishes to validate an irradiation process for several spices: basil (herb), cumin (whole seed), black pepper (whole berry), black pepper (ground), marjoram (herb), celery (whole seed), and white pepper (whole berry).

The processor would group the materials based on their part of the plant and product form. The herb group would be basil and marjoram, the berry group would be black and white pepper, and the seed group would be celery and cumin. Within the berry group there are whole and ground products. Ground black pepper would be a separate group from whole black and white pepper. The processor would then select the highest density product from each group. In this case it would be basil, cumin, ground black pepper, and white pepper. Once the process is validated for these spices it would be assumed to be validated for other spices with similar physical characteristics – i.e., for other spices in the same group.

Example 3. If a processer wishes to validate a process for a group of spices, it may be helpful to develop a table summarizing the key parameters of interest. For example, in the table below, two fruit spices are listed: black pepper and paprika powder, along with measures for density and water activity.

	Critical Parameters for Processes		
Spice	**Part of Plant**	**Density (g/L)**	**Water Activity (a_w)**
Black Pepper	Fruit	358.4	0.6
Paprika Powder	Fruit	459.9	0.45

In this example, the paprika has the higher density and lower water activity and should be used as the "worst case" scenario. Neither of the spices is this example have known microbial inhibitory properties. If a spice in the grouping is known to have inhibitory properties, it may be prudent to select another spice if a steam process is being used.

Example 4. In some situations, it may not be immediately obvious which commodity should be considered the "worst-case" upon which to base the validation for the group. For example, in

the table of leafy herbs list below, different spices represent the worst-case for different parameters. As an example, the "worst-case" (highest) for density is rosemary, while the "worst-case" (lowest) for water activity is dill weed.

Part of plant	Spice	Bulk density (g/L)	Water Activity (approximate)	Reference
Leaf	Bay leaves	121.7	0.46-0.52	Aktaşa et al., 2015
Leaf	Dill weed	209.7	0.10 – 0.20	Kathirvel et al., 2006
Leaf	Marjoram	115.0	0.32	Sarabandi et al., 2018
Leaf	Oregano	202.9	**0.55**	Voelker et al., 2020
Leaf	Parsley	108.2	0.32	Voelker et al., 2020
Leaf	Rosemary	223.2	0.50	Voelker et al., 2020
Leaf	Sage	135.3	0.23-0.29	Sahin-Nadeem et al., 2013
Leaf	Spearmint	108.2	0.23-0.38	Kathirvel et al., 2006
Leaf	Sweet basil leaf	142.0	0.50	Voelker et al., 2020
Leaf	Tarragon	121.7	0.33-0.36	Koç et al., 2018
Leaf	Thyme	182.6	0.50	Voelker et al., 2020

When selecting the representative worst-case spice, first and foremost, consideration should be given to the parameters of most importance to the treatment method. For example, if the product is being irradiated, then density may be the most important parameter, while water activity may be more relevant to a thermal process. The influence of product density on irradiation efficacy is well-detailed, as it impacts the absorbed dose (Sanyal et al., 2017; WHO, 1988).

If the most important parameter is unknown for the treatment method being used, this situation may be handled several ways. The manufacturer may choose to validate two different spices for the group, or the group could be broken into subgroups with more clear candidates. Another option may be for the manufacturer to manipulate the density and water activity of one of the candidates to have the "worst-case" parameters of the other. For example, by grinding the product to a finer grind for the purposes of creating an even more worse case example of the product for conducting the study.

Summary of Examples

When an intervention process to control microbial contamination is being validated, it is necessary to evaluate the process under the most extreme conditions that may occur. That is for a group of spices, the product with the physical characteristics that would most favor the survival or resistance of the target microorganism must be evaluated. Depending on the source and environmental conditions, the water activity could also vary. The physical characteristics

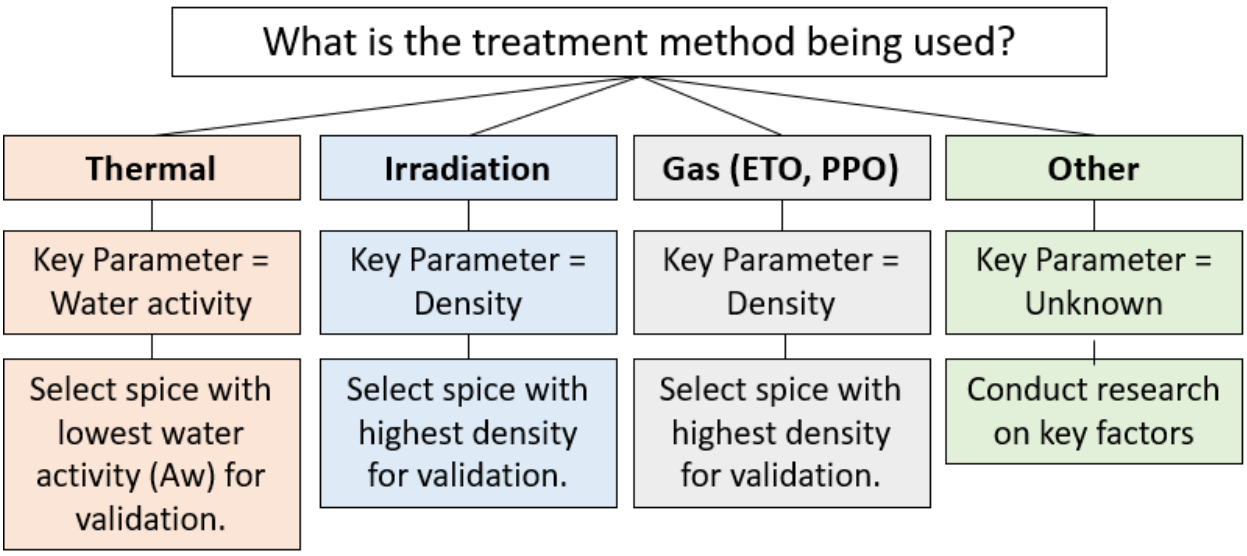
within a specific spice should be considered (whole vs. ground as well as physical size), as milling or grinding will affect the product density. The key point is "within a specific spice" as different spices will have different densities. As an example, ground cinnamon and whole pepper may have the same density, but a direct comparison between the two, based solely on density, may not be appropriate.

In validating a process for several spices, the processor should consider the guidance provided in Table 5 for the properties of the spice(s) and Appendix 2 for the properties of the process. An outline of a final report, which is essential to documenting the validation, is given in Appendix 3.

Table 5. Process to Validate Groupings of Spices

Overview of the Process to Validate Groupings of Spices	
Step 1.	Develop groupings based on similarities of products (e.g., part of the plant).
Step 2.	Determine critical parameters for treatment method (e.g., density, water activity) (Figure 1). This assessment should be based on the scientific evidence, as summarized in the literature of through experimental studies.
Step 3.	Identify a "worst-case" scenario product or products for each group, based on the critical factors for each grouping.
Step 4.	Run a challenge study for the worst-case product(s) from each group.

Figure 1. Determination of critical paratmers for a treatment method.*



**Note: If steam treatment or wet inoculation is being used, spices with known inhibitory properties should be avoided to prevent the potential for erroneous results.*

Conclusions

1) The physical properties of the spices should be taken into consideration in determining the “worst-case” scenario. This should include lot to lot variation.

2) The parameters associated with the process should also represent the “worst-case” scenario for the treatment method being used, which would include the minimal parameters expected during processing (e.g., lowest temperature, steam pressure, relative humidity or exposure time for the process).

Cited References

Abdel-Khalek, H.H. 2008. Effect of gamma irradiation on the microbial, chemical quality and the biological activity of some spices and herbs. Thesis. https://inis.iaea.org/collection/NCLCollectionStore/_Public/40/024/40024286.pdf?r=1

Acuff, J.C., Wu, J., Marik, C., Waterman, K., Gallagher, D., Huang, H., Williams, R.C. and Ponder, M.A. 2020. Thermal inactivation of *Salmonella*, Shiga toxin-producing *Escherichia coli, Listeria monocytogenes*, and a surrogate (*Pediococcus acidilactici*) on raisins, apricot halves, and macadamia nuts using vacuum-steam pasteurization. International Journal of Food Microbiology, 333, p.108814.

Aktaşa, M., S. Sevik, M. Bahadır Özdemira, and E. Gönen. 2015. Performance analysis and modeling of a closed-loop heat pump dryer for bay leaves using artificial neural network. Applied Thermal Engineering 87(5):714-723.

Alamn, M.K., Choudhuryn, N. Chowdhury, N.A., and Youssouf, Q.M. 1992. Decontamination of spices by gamma radiation. Letters in Applied Microbiology. 14:199-202

American Spice Trade Association. 2019. ASTA Report on Surrogate Selection and Proposed Spice Groupings for Validation Studies.

American Spice Trade Association. 2016. ASTA Spice List.

Archer, J., Jervis, E. T., Bird, J., and Gaze, J. E., 1998. Heat resistance of *Salmonella* Weltevreden in low-moisture environments. Journal of Food Protection. 61, 969–973.

Arias-Rios, E.V., G.R. Acuff, A. Castillo, L.M. Lucia, S.E. Niebuhr, and J.S. Dickson. 2019. Identification of a surrogate to validate irradiation processing of selected Spices. LWT - Food Science and Technology 102:136–141.

Billing, J. and Paul W. Sherman. 1998. Antimicrobial Functions of Spices: Why Some Like it Hot. The Quarterly Review of Biology, Vol. 73, No. 1 (Mar., 1998), pp. 3-49.

Bowman, L.S., K.M. Waterman, R.C. Williams, and M.A. Ponder. 2015. Inoculation preparation affects survival of Salmonella enterica on whole black peppercorns and cumin seeds stored at low water activity. Journal of Food Protection, 78(7):1259-1265.

Ceylan, E. and Fung, D.Y. 2004. Antimicrobial activity of spices 1. Journal of Rapid Methods & Automation in Microbiology, 12(1), pp.1-55.

Chen, L., Wei, X., Chaves, B. D., Jones, D., Ponder, M. A., and Subbiah, J. 2021. Inactivation of *Salmonella enterica* and *Enterococcus faecium* NRRL B2354 on cumin seeds using gaseous ethylene oxide. Food Microbiology, 94, 103656.

Eiss, M.I. 1984. Irradiation of Spices and Herbs. Food technology in Australia 36: 362-370.

Farkas, J., & Andrassy, E. 1988. Comparative analysis of spices decontaminated by ethylene oxide or gamma radiation. Acta Alimentaria, 17(1), 77-94.

Food and Drug Administration (FDA). 2017. Risk Profile: Pathogens and Filth in Spices. https://www.fda.gov/media/108126/download .

Golden, C.E., M. E. Berrang, W. L. Kerr, and M. A. Harrison. 2019. Slow-release chlorine dioxide gas treatment as a means to reduce *Salmonella* contamination on spices. Innovative Food Science and Emerging Technologies, 52 (2019) 256–261

Hildebrandt, I.M., C. Hu, E.M. Grasso-Kelley, P. Ye, N.M. Anderson, S.E. Keller. 2017. Dry transfer inoculation of low-moisture spices containing antimicrobial compounds. Journal of Food Protection, 80(2):338-344.

International Atomic Energy Agency (IAEA). 2015. Manual of good practice in food irradiation. Sanitary, phytosanitary and other applications. Technical Report Series No. 481. Vienna, Italy.

Jeong, S., Marks, B.P., Ryser, E.T., and Harte, J.B. 2012. The effect of X-ray irradiation on *Salmonella* inactivation and sensory quality of almonds and walnuts as a function of water activity. International Journal of Food Microbiology, 153(3): 365-371.

Juri, M.L., Ito, H., Watanabe, H., and Tamura, N. 1986. Distribution of Microorganisms in Spices and Their Decontamination by Gamma-irradiation. Agricultural and Biological Chemistry, 50 (2), 347-355.

Kathirvel, K., K. R. Naik, Y. Gariepy, V. Orsay and G.S.V. Raghavan. 2006. Microwave Drying - A promising alternative for the herb processing industry. Annual Conference of the Canadian Society for Bioengineering paper 06-212 https://elibrary.asabe.org/pdfviewer.asp?param1=s:/8y9u8/q8qu/tq9q/5tv/L/s8ruIGGM/GMIHI.5tv&param2=I/N/IGII&param3=HIP.HOM.ILH.LI&param4=22132

Kereluk, K., Gammon, R.A., and Lloyd, R.S. 1970. Microbiological aspects of ethylene oxide sterilization. III. Effects of humidity and water activity on the sporicidal activity of ethylene oxide. Applied Microbiology, 19(1): 157-162.

Koç, B., N. Çağlar, and G. Atar. 2018. Functional properties of dried tarragon affected by drying method. IDS'2018 – 21st International Drying Symposium València, Spain, 11-14 September

2018. DOI: http://dx.doi.org/10.4995/ids2018.2018.7834.
https://riunet.upv.es/bitstream/handle/10251/117775/7834-22503-3-PB.pdf?sequence=1&isAllowed=y

Liu, S., Tang, J., Tadapaneni, R.K., Yang, R., and Zhu, M-J. 2018. Exponentially increased thermal resistance of *Salmonella* spp. And *Enterococcus faecium* at reduced water activity. Applied Environmental Microbiology. 2;84(8):e02742-17.

Molnár, H., Bata-Vidács, I., Baka, E., Cserhalmi, Z., Ferenczi, S., Tömösközi-Farkas, R., Adányi, N. and Székács, A. 2018. The effect of different decontamination methods on the microbial load, bioactive components, aroma and colour of spice paprika. Food Control, 83, pp.131-140.

Newkirk, J.J., J. Wu, J. C. Acuff, C. B. Caver, K. Mallikarjunan, B. D. Wiersema, R. C. Williams and M. A. Ponder. 2018. Inactivation of *Salmonella enterica* and Surrogate *Enterococcus faecium* on Whole Black Peppercorns and Cumin Seeds Using Vacuum Steam Pasteurization. Frontiers in Sustainable Food Systems, 2:48. doi: 10.3389/fsufs.2018.00048

Ozturk, S., F. Kong, R. K. Singh, J. D. Kuzy, C. Li and S. Trabelsi. 2018. Dielectric properties, heating rate, and heating uniformity of various seasoning spices and their mixtures with radio frequency heating. Journal of Food Engineering, 228:128-141

Park, H.W., Xu, J., Balasubramaniam, V.M., and Snyder, A.B. 2021. The effect of water activity and temperature on the inactivation of *Enterococcus faecium* in peanut butter during superheated steam sanitation treatment. Food Control 125, 107942. https://doi.org/10.1016/j.foodcont.2021.107942

Sahin-Nadeem, H., C. Dinçer, M. Torun, A. Topuz, and F. Özdemir. 2013. Influence of inlet air temperature and carrier material on the production of instant soluble sage (Salvia fruticosa Miller) by spray drying. LWT – Food Science and Technology, 52:31-38

Sandle, T. 2017. Validation requirements for gaseous sterilization using ethylene oxide. *JVT Network – Institute for Validation Technology.*

Sanyal, B., Prakasan, V., Chawla, S.P., and Ghosh, S.K. 2017. A study to assess the role of bulk density of process load in "6"0Co based food irradiation facility. BARC Newsletter, p. 17-19. International Atomic Energy Agency.

Sarabandi, K., A. Sadeghi Mahoonak, and M. Akbari. 2018. Physicochemical properties and antioxidant stability of microencapsulated marjoram extract prepared by co-crystallization method. Journal of Food Process Engineering, 2019;42:e12949. https://doi.org/10.1111/jfpe.12949

Shah, M.K., G. Asa, J. Sherwood, K. Graber and T.M Bergholtz. 2017. Efficacy of vacuum steam pasteurization for inactivation of *Salmonella* PT 30, *Escherichia coli* O157:H7 and *Enterococcus faecium* on low moisture foods. International Journal of Food Microbiology, 244:111–118.

Shelef, L.A., 1984. Antimicrobial effects of spices 1. Journal of food safety, 6(1):29-44.

Steinbrunner, P.J., Limcharoenchat, P., Suehr, Q., Ryser, E.T., Marks, B.P., and Jeong, S. 2019. Effect of Food Structure, Water Activity, and Long-Term Storage on X-Ray Irradiation for Inactivating *Salmonella* Enteritidis PT30 in Low-Moisture Foods. Journal of Food Protection, 82(8):1405-1411.

Syamaladvi, R.M., Tang, J., Villa-Rojas, R., Sablani, S., Carter, B., and Campbell, G. (2016) Influence of water activity on thermal resistance of microorganisms in low-moisture foods: a review. Comprehensive Reviews in Food Science and Food Safety, 15: 353-370.

Toofanian, F., and Stegeman, H. (1988). Comparative effect of ethylene oxide and gamma irradiation on the chemical, sensory and microbial quality of ginger, cinnamon, fennel and fenugreek. Acta Alimentaria, 17(4), 271-281.

Vajdi, M. and Pereira, R.R. 1973. Comparative effects of ethylele oxide, gamma irradiation and microwave treatments on selected spices. Journal of Food Science. 38:893-895.

Van Doren, J.M., K.P. Neil, M. Parish, L. Gieraltowski, L. H. Gould, and K. L. Gombas. 2013. Foodborne illness outbreaks from microbial contaminants in spices,1973-2010. Food Microbiology 36:456-464.

Voelker, A.L., Sommer, A. A. and Mauer, L.J. 2020. Moisture sorption behaviors, water activity-temperature relationships, and physical stability traits of spices, herbs, and seasoning blends containing crystalline and amorphous ingredients. Food Research International, 136:109608.

Wei, X., T. Verma, M-G. C. Danao, M.A. Ponder and J. Subbiah. 2021. Gaseous chlorine dioxide technology for improving microbial safety of spices Innovative Food Science and Emerging Technologies 73:102783

Wei, X., Chen, L., Chaves, B. D., Ponder, M. A., & Subbiah, J. (2021). Modeling the effect of temperature and relative humidity on the ethylene oxide fumigation of *Salmonella* and *Enterococcus faecium* in whole black peppercorn. LWT, 140, 110742.

World Health Organization (WHO). 1988. Food irradiation: a technique for preserving and improving the safety of food. World Health Organization, Geneva, 1988.

Zaied, S.E.A., Aziz, N.H. and Ali, A.M. 1996. Comparing effects of washing, thermal treatments and gamma irradiation on quality of spices. Nahrung 40 Fir. I , S. 32-36.

Zaman, S., Md. Khorshed Alam, Md. Firoz Mortuza, and Md. Latiful Bari. Effectiveness of Irradiation Treatment in Eliminating *E. coli* O157:H7 and *Salmonella* in Dried Organic Herb Samples Intended for Use in Blended Tea. Journal of Food and Nutrition Sciences. Special Issue: Food Processing and Food Quality, Vol. 3, No. 1-2, 2015, pp. 165-170.

Appendix 1. Additional Resources

1. American Spice Trade Association. 2001a. General Protocol for the Validation of Microbiocidal Processes on Pathogen Contaminated Spices and Culinary Herbs - CHAMBER TREATMENT

2. American Spice Trade Association. 2001b. General Protocol for the Validation of Microbiocidal Processes on Pathogen Contaminated Spices and Culinary Herbs - GAMMA IRRADIATION

3. American Spice Trade Association. 2001c. General Protocol for the Validation of Microbiocidal Processes on Pathogen Contaminated Spices and Culinary Herbs - STEAM TREATMENT

4. American Spice Trade Association. 2013. ASTA White Paper on Validation of Microbial Reduction Processes for Spices. https://www.astaspice.org/download/1492

5. American Spice Trade Association. 2017. ASTA Webinar Series on Validation of Microbial Reduction Processes. https://www.astaspice.org/industry-news-events/webinars/past-webinars-for-download/asta-webinar-series-validation-microbial-reduction-processes/

6. ASTM. 2010. Standard Guide for Irradiation of Dried Spices, Herbs, and Vegetable Seasonings to Control Pathogens and Other Microorganisms. F1885 − 04 (Reapproved 2010).

7. Codex Alimentarius Commission. 2003a. General Standard for Irradiated Foods. CODEX STAN 106-1983, Rev.1-2003. https://www.fao.org/fao-who-codexalimentarius/sh-proxy/en/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FStandards%252FCXS%2B106-1983%252FCXS_106e.pdf

8. Codex Alimentarius Commission. 2003b. Code of Practice for Radiation Processing of Food. (CAC/RCP 19-1979). https://www.fao.org/fao-who-codexalimentarius/sh-proxy/en/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FStandards%252FCXC%2B19-1979%252FCXP_019e.pdf

9. Codex Alimentarius Commission. 2008. Guidelines for the Validation of Food Safety Control Measures. https://www.fao.org/fao-who-codexalimentarius/sh-

proxy/ar/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FStandards%252FCXG%2B69-2008%252FCXG_069e.pdf

10. Food and Drug Administration (FDA). 2011. Guidance for Industry - Process Validation: General Principles and Practices. https://www.fda.gov/media/71021/download

11. International Standard Organization (ISO). 2011. Food Irradiation – Requirements for the development, validation and routine control of the process of irradiation using ionizing radiation for the treatment of food. ISO 14470.

12. International Standard Organization (ISO). 2014. Sterilization of health-care products — Ethylene oxide — Requirements for the development, validation and routine control of a sterilization process for medical Devices. ISO 11135.

13. International Standard Organization (ISO). 2021. Microbiology of the food chain — Requirements and guidelines for conducting challenge tests of food and feed products — Part 2: Challenge tests to study inactivation potential and kinetic parameters. ISO 20976-2 (draft).

14. National Advisory Committee on Microbiological Criteria for Foods, Parameters for Determining Inoculated Pack/Challenge Study Protocol. Journal of Food Protection, 2010, Vol. 73, No. 1, pp. 140–202.

Appendix 2. Properties of process - Guidance for determining conditions for a validation of an antimicrobial process for spices.

Question Number		Question
1		What is the process?
	1	Steam
	2	Gas (e.g., Ethylene oxide, propylene oxide)
	3	Irradiation
	4	Alternative processes
		Steam
	1a	What is the minimum product temperature?
	1b	What is the minimum pressures?
	1c	What is the minimum exposure time?
	1d	What is the minimum vaccum applied (if used)?
		Gas
	2a	What is the spice with the highest density?
	2b	What is the minimum ethylene oxide concentration?
	2c	What is the lowest relative humidity in the process?
	2d	What is the minimum pressure?
	2e	What is the shortest exposure time?
	2f	What is the lowest/highest product temperature in the process?
		Irradiation
	3a	What is the spice with the highest density?
	3b	What is the minimum absorbed dose?
		Alternative Processes
	4a	What are the paramters that impact the effect of the process?
	4b	e.g. Dielectric properties, heating rate, and heating uniformity

Appendix 3. Outline of a hypothetical validation experiment. (Following the general guidance of ISO 20976-1).

Step 1: Identify the Process to be Validated

- Determine which process(s) (steam, gas, irradiation or alternative process) are to be evaluated.

Step 2: Identify the critical spice parameters associated with the process.

- For each process, determine what the critical spice parameters are for the process (see "Considerations for Process Validations Based on Groupings")

Step 3: Select the spices to be evaluated and their critical properties.

- Determine which spices to evaluate
- Group the spices based on "Considerations for Process Validations Based on Groupings."
- From each initial group, select two spices for further evaluation, based on the parameters critical to the process.

Step 4: Design the validation

- The validation study should be carried out in accordance with generally accepted principles of validation. Please refer to the references in the additional resources, especially the appropriate ASTA documents (1 -3), the FDA Guidance (8), ISO 20976-2 (11) and the National Advisory Committee (12).

Step 5: Prepare the report.

- Although there is no required format for the report, it should contain the following information presented in clear and unambiguous language.

I. Title, to clearly state what was evaluated
   - Include specific process
II. Objective of the study, type of challenge test and target reduction level
   - State minimum target bacterial reduction
III. Experimental protocol
   - All rationale for the selection of the spices, inoculation procedures and process
IV. Sample analysis
   - The process being evaluated is inhibitory. Sample analysis must use methods capable of recovering injured bacteria.
V. Results
   - All sample results should be included in an Appendix. The summary of results should indicate all calculations and statistical methods used.
VI. Conclusions

- What were the results? What would a reasonable person objectively examining the study conclude?

VII. Reference documents

- All reference documents used to support the selection of spices, the experimental protocol, sample analytical methods and analysis of results.