ASTA Report on Surrogate Selection and Proposed Spice Groupings for Validation Studies

February 2019

Executive Summary: The American Spice Trade Association (ASTA) commissioned research to determine an appropriate surrogate organism for use in spice validation studies and to group spices based on having a similar degree of microbial inhibition. Based on laboratory research, *Enterococcus faecium* was determined to be an appropriate surrogate organism to validate *Salmonella enterica* control in microbial reduction processes for spices. Spice groupings were determined based on the degree of microbial inhibition reported in the published literature.

Background: The U.S. Food and Drug Administration (US FDA) has focused on microbiological contamination of spices for several years. The Preventive Controls for Human Food (PCHF) rule, adopted under the FDA Food Safety Modernization Act (FSMA), requires food companies to develop a food safety plan. As part of this plan, companies must identify hazards requiring preventive controls (e.g., *Salmonella*) and implement preventive controls to mitigate these hazards. The PCHF rule requires validation of process preventive controls, meaning spice companies are required to validate pathogen reduction processes used to control *Salmonella*.

The spice industry has historically relied on various microbial reduction processes, including heat (steam), gas, and irradiation. The US FDA has challenged the industry to provide scientific data which shows that these treatment processes accomplish the intended objective, a 5 log_{10} reduction in the population of a potential pathogen, with a particular focus on non-typhoidal *Salmonella enterica*.

The typical population of *Salmonella enterica*, when it occurs, is significantly less than 100,000 colony forming units per gram. To demonstrate a 5 log_{10} reduction of non-typhoidal *Salmonella enterica* in a spice, a processor would have to inoculate the spice with non-typhoidal *Salmonella enterica* to a population of greater than 100,000 colony forming units per gram. Since the deliberate introduction of a human pathogen into a commercial food processing establishment is ill-advised, the alternative is to identify a non-pathogenic bacterium (surrogate) that responds in a similar manner to the microbial reduction process.

The US FDA has raised a concern about the use of surrogates specifically with spices. Spices in general have inherent antimicrobial properties, and the concern relates to the potential that a surrogate may be inhibited by the spice to a greater degree than non-typhoidal *Salmonella enterica*. If this were in fact true, the microbial reduction estimated by the surrogate in response to an antimicrobial process could be greater than the actual reduction of the pathogen, because of the differences in inhibition between the surrogate and the pathogen due to the inherent nature of the spice.

The US FDA recommended that ASTA develop data demonstrating the relative inhibition of the proposed surrogate, *Enterococcus faecium*, and a group of non-typhoidal *Salmonella enterica*. The US FDA has also suggested that they will not require validation data to be collected for each and every spice individually. Rather, FDA agreed in principle to the approach of separating the spices into groupings. A validated process for one spice in the group would be considered representative of all of the spices in the group.

Data Request 1: Provide data demonstrating the relative inhibition of the proposed surrogate, *Enterococcus faecium*, and a group of non-typhoidal *Salmonella enterica* by individual spices.

At US FDA's request, ASTA had research conducted to evaluate four spices for inhibitory action against *Salmonella enterica* and *Enterococcus faecium*. The spices specifically identified by US FDA for research were:

- Allspice (berry)
- Cinnamon (bark)
- Cloves (bud)
- Oregano (leaf)

These four spices are representative of spices that could have the greatest inhibitory impact on microorganisms, both pathogenic and surrogates. The inhibitory effect of these four spices was measured against five different strains of *Salmonella enterica* (three of which were obtained from US FDA and were isolated from spices), and *Enterococcus faecium*. The inhibitory effect was assessed by a standard disk-diffusion assay, where zones of inhibition were measured. The interpretation of the results of this assay can be summarized as: smaller zones of inhibition indicate less inhibition, larger zones indicate greater inhibition.

The results of the ASTA study show that the zones of inhibition for the surrogate *Enterococcus faecium* were either statistically not different from, or statistically smaller (less inhibition) than, the zones of inhibition for the five strains of non-typhoidal *Salmonella enterica*.
The results of the statistical analysis of the zones of inhibition for all four spices are given in Table 1. *Enterococcus faecium* had the smallest zone of inhibition for all four

spices, indicating that it was inhibited less by these four spices than the five strains of non-typhoidal *Salmonella enterica*.

The conclusion from these experiments is that for the specific spices tested in this experiment, the proposed surrogate *Enterococcus faecium* was inhibited by the spices to the same degree, or less than, the five strains of non-typhoidal *Salmonella enterica* tested.

Data Request 2: Propose a method of grouping spices.

There are at least two approaches to grouping spices in regard to validating microbial interventions. The first is to group by the part of the plant from which the spice is derived, while the second takes into account the inherent antimicrobial properties within the spice itself. It is the consensus opinion of ASTA that the most relevant characteristic of spices for the purpose of validating microbial reduction processes is their ability to inhibit microorganisms. ASTA met with FDA in 2017 to discuss the idea of developing groupings of spices for use in validation studies. FDA officials agreed to the concept of groupings and recommended that ASTA develop a technical justification for their basis.

The ability to inhibit microorganisms is relevant to both the pathogenic bacteria in question (especially *Salmonella enterica*) and proposed surrogate bacteria, including *Enterococcus faecium*. Billing and Sherman (1998) published a review on spices that included an evaluation of their antimicrobial potential (Figure 1). The results of this review can be used to categorize the 30 spices (including garlic and onion) into 3 categories (>75% inhibition, 50%-75% inhibition, and <50% inhibition), based on the spice's inherent ability to inhibit microorganisms (Table 2).

The American Spice Trade Association (2016) has a "spice list", which identifies the part of the plant from which the individual spice is derived. The list is summarized in Table 3. The ASTA list categorizes 51 spices into nine different categories, based on the part of the plant from which the spice is derived.

Table 4 lists the spices that appear on the ASTA (2016) list and are not included in the Billing and Sherman (1998) paper. With the exception of mace (the only spice derived from the plant's aril), all of the categories in the ASTA list were represented in the Billing and Sherman paper. As a default position, in the absence of other data, the spices lacking specific inhibition data could be conservatively assumed to be in the category of "most inhibitory" spices, those with inhibition of more than 75%.

Conclusions:

1) The currently available data suggests that the inhibitory effect of the spices tested by ASTA support the use of *Enterococcus faecium* as a surrogate for process validation. This is based on the inhibitory effects of the spices on five strains of non-typhoidal *Salmonella enterica* tested and the proposed surrogate *Enterococcus faecium. Enterococcus faecium* was inhibited to the same degree, or less than, the five strains of non-typhoidal *Salmonella enterica* tested.

2) Since *Enterococcus faecium* is inhibited to the same degree or less than the five strains of non-typhoidal *Salmonella enterica* tested with spices from the most inhibitory group of spices (>75% inhibition), it would not be expected to be inhibited to a greater degree by spices in the lesser inhibitory groups (75% to 50%, <50%). This means that *Enterococcus faecium* can be used as a surrogate for all spices, based on inhibition.

3) Based on published research, *Enterococcus faecium* can be used as a surrogate bacterium to validate microbial interventions of spices relating to *Salmonella* (Arias-Rios et al., 2019; Newkirk et al., 2018; Shah et al., 2017).

4) A logical approach to grouping the spices, in terms of potential validation procedures, would be to group them by inhibitory potential.

5) The proposed spice groupings are listed in Table 5.

Table 1. Zones of inhibition for *S. enterica* and *E. faecium* for Allspice, Oregano, Cinnamon and Cloves

Spice	Bacterium	Zone of Inhibition
Allspice	Sal1436	11.5^{A}
	Sal2846	10.26^{A}
	Sal2867	9.0^{A}
	Sal9150	17.0^{A}
	Sal35640	10.34^{A}
	Enterococcus faecium	7.97^{A}
Oregano	Sal1436	$11.97^{A,B}$
	Sal2846	$14.1^{A,B}$
	Sal2867	$13.32^{A,B}$
	Sal9150	$15.18^{A,B}$
	Sal35640	17.12^{A}
	Enterococcus faecium	10.9^{B}
Cinnamon	Sal1436	16.67^{A}
	Sal2846	17.1^{A}
	Sal2867	$14.7^{A,B}$
	Sal9150	$14.6^{A,B}$
	Sal35640	$15.34^{A,B}$
	Enterococcus faecium	$6.64^{A,B}$
Cloves	Sal1436	11.5^{A}
	Sal2846	12.11^{A}
	Sal2867	18.63^{A}
	Sal9150	12.52^{A}
	Sal35640	10.82^{A}
	Enterococcus faecium	11.55^{A}

Zones of inhibition with different superscripts within spice type are significantly ($P<0.05$) different.

Table 2. Antimicrobial Properties (Inhibition of growth or killing) of 30 spices (Billing and Sherman, 1998).

75% Inhibition or greater	50% to 75% inhibition	<50% Inhibition
Garlic, onion, allspice oregano, thyme, cinnamon, tarragon, cumin, cloves, lemon grass, bay leaf, capsicums, rosemary, marjoram, mustard	Caraway, mint, sage, fennel, coriander, dill, nutmeg, basil, parsley	Cardamon, pepper (black/white), ginger, anise seed, celery seed, lemon/lime

Table 3. Spices categorized by the part of the plant that they are derived from (ASTA, 2016).

Part of Plant	Spice
Aril	Mace
Bark	Cinnamon
Berry	Allspice, juniper, pepper (black, white, green, pink)
Bud	Cloves
Flower	Chamomile (English, Roman, German, Hungarian), lavender,
Fruit	Anise (star), capsicums, cardamom, paprika, vanilla
Leaf	Balm (lemon), Basil leaf (sweet), Bay leaves, chervil, chives, cilantro, dill weed, marjoram, oregano, parsley, peppermint, rosemary, sage, savory, spearmint, tarragon, thyme
Root	Galangal, ginger, horseradish
Seed	Anise seed, black caraway (Russian, Black cumin), caraway seed, celery seed, coriander, cumin seed, dill seed, fennel seed, fenugreek seed, mustard seed, nutmeg, poppy seed, sesame

Table 4. Spices categorized by the part of the plant that they are derived from (ASTA, 2016) which are not included in the Billing and Sherman (1998) review.

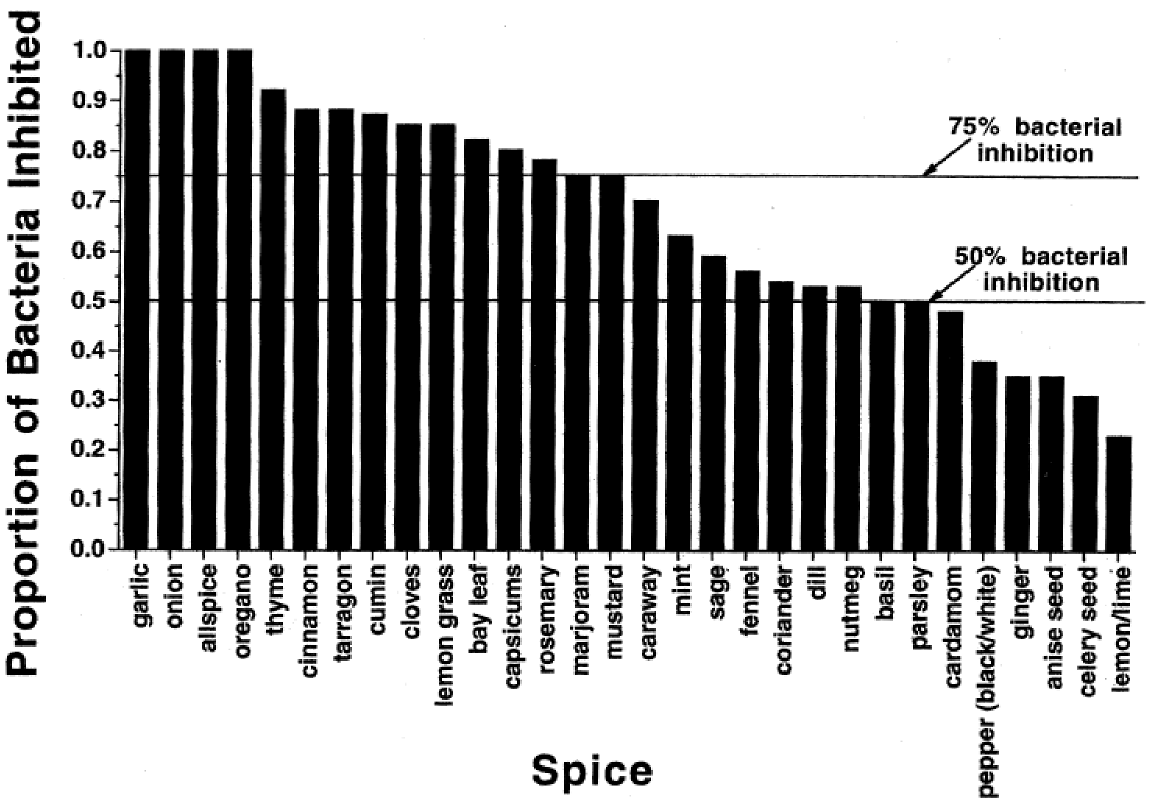
Part of Plant	Spice
Aril	Mace
Bark	
Berry	Juniper
Bud	
Flower	Chamomile (English, Roman, German, Hungarian), lavender
Fruit	Paprika, vanilla
Leaf	Balm (lemon), chervil, chives, cilantro, peppermint, savory, spearmint
Root	Galangal, horseradish
Seed	Fenugreek seed, poppy seed, sesame

Table 5. Proposed spice groupings for validation studies.

Degree of Inhibition	Proposed Group Number	List of Spices	Spices Included by Default[1]
Most Inhibitory (>75%)	1	Garlic, onion, allspice oregano, thyme, cinnamon, tarragon, cumin, cloves, lemon grass, bay leaf, capsicums, rosemary, marjoram, mustard	Mace, Juniper, Chamomile (English, Roman, German, Hungarian), lavender Paprika, vanilla Balm (lemon), chervil, chives, cilantro, peppermint, savory, spearmint, Galangal, horseradish, Fenugreek seed, poppy seed, sesame
Intermediate Inhibition (50% to 75%)	2	Caraway, mint, sage, fennel, coriander, dill, nutmeg, basil, parsley	
Less Inhibition (<50%)	3	Cardamon, pepper (black/white), ginger, anise seed, celery seed, lemon/lime	

1 In the absence of other data, the spices which are included in the ASTA list but not in the Billing and Sherman (1998) paper are placed in the “most inhibitory” category.

Figure 1. Antimicrobial Properties (Inhibition of growth or killing) of 30 spices for which appropriate data were available, arrayed from greatest to least inhibition.



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