Microbiology of Spices

Date of Last Revision: April 13, 2021

Revision No. 3

Supersedes: 2013

Reason for Revision: Periodic Review

Table of Contents

Introduction

Many spices are grown in tropical climates where they are subjected to excess heat, humidity, and minimal good agricultural practices. These conditions may lead to contamination by microorganisms and their subsequent growth. There are microorganisms indigenous to the soil, but other sources of contamination include dust and dirt, insects, and fecal materials from birds, rodents and other animals. Therefore, raw unprocessed spices commonly harbor large numbers of bacteria and fungi, including organisms that cause spoilage and food borne pathogens such as *Salmonella*. As such, it is important that the spice industry understands the extent of microbial contamination in spice commodities and develops programs that minimize the risk for contamination during growing, harvesting, drying, transport, processing and post-processing storage. Additionally, companies should have in place validated kill steps and comply with all relevant laws and regulations to ensure the safety of the product.

Pathogens of Concern in Spices

A number of pathogens that pose a risk to human health, including *Salmonella, E-coli*, *C. perfringens* and *B. cereus* may be found on spices. These microorganisms may contaminate the spices during cultivation, harvest, and storage. The risk of microbial contamination is also influenced by a number of factors including the nature of each spice, part of the plant, naturally occurring microbial activity, surrounding environment (e.g. soil, wind, water, flora and fauna), and local growing conditions (e.g. temperature humidity, rain and available sunlight). As such, it is critical that spice companies have good agricultural, harvesting, and manufacturing practices as well as validated treatment methods to control microbial contamination [1].

Although there is a known risk of pathogens occurring on spices, the total number of outbreaks attributed to contaminated spices in the last 30 years is low. This may in part be due to the low water activity of dried spices, which prevents the growth and viability of most non-sporeformers, consequently limiting the chance of illness caused by these organisms. The vast majority of outbreaks attributed to dried spices have been contaminated with *Salmonella*. This is likely due to the fact that some strains of *Salmonella* are particularly adaptable to dry conditions [2]. However, outbreaks can be associated with other pathogenic species.

The relatively low number of outbreaks may also in part be attributed to the role of spices as ingredients, and the extensive use of treatments such as irradiation, ethylene oxide, and steam treatments among others to control pathogens. The subsequent sections summarize evidence on the presence of pathogens in spices as well as notable outbreaks and food safety incidents. Further information on foodborne illness and recalls of spices may be found in [3-5].

1. Salmonella spp.

Salmonella is the most common bacterial pathogen associated with product recalls and outbreaks in spices [4, 6]. *Salmonella* can present a human health hazard even when detected in low numbers

when no kill step is applied prior to consumption. *Salmonella* is very stable in dry environments and can survive through production, distribution, and eventually consumption.

An FDA study of retail spices, published in 2017, found the following incidence of *Salmonella* [7].

Table 1. Estimated Salmonella prevalence in 125 g of spices offered for sale at retail establishments.

Spice Type	Total Number of Samples Tested	Number of Samples Positive for Salmonella	Salmonella Prevalence
Basil	529	1	0.19
Black pepper	1,264	3	0.24
Coriander (ground)	543	3	0.56
Cumin	549	0	0.00
Curry powder (ground)	518	1	0.19
Dehydrated garlic (ground)	615	3	0.49
Oregano	669	1	0.15
Paprika (ground)	816	2	0.25
Red pepper (ground)	633	4	0.64
Sesame seed (whole)	526	0	0.00
White pepper (ground)	588	0	0.00

Below is a summary of *Salmonella* outbreaks, reportedly due to contaminated dried herbs and spices that have been recorded in the general literature in the last 50 years.

- 1974, Canada
  An outbreak associated with white pepper contaminated with *Salmonella* weltevreden was reported [8].

- 1984, Norway
  In Norway, black pepper contaminated with *Salmonella* oranienburg was implicated in an outbreak affecting 126 individuals [9].

- 1995, Germany
  Foods - potato chips and other snack foods - prepared with paprika contaminated with at least three serovars of *Salmonella* (*S. saintpaul, S. rubislaw*, and *S. javiana*) were implicated in an estimated 1000 illnesses in Germany [10]. Considered the largest documented outbreak due to

contaminated spices, this event demonstrated that *Salmonella* adapted to the dried state were capable of causing illness even in very low numbers: 0.04 - 0.45 organisms per gram.

- 2007, United States
  In the United States, there was a *Salmonella* Wandsworth outbreak associated with a seasoning mix consisting of broccoli powder, parsley powder, and other spices used to coat a snack puff after the final food manufacturing pathogen reduction step [11]. The only ingredient which tested positive for *S*. Wandsworth was the broccoli powder, although testing also found *Salmonella* Typhimurium in the seasoning mix and *Salmonella* Mbandaka from parsley powder. Human illnesses from the *Salmonella* Typhimurium were also linked to the spice covered snack puffs, but there were no human cases of *Salmonella* Mbandaka.

- 2008-2009, United States
  There was an outbreak of *Salmonella* Rissen in the United States that implicated white pepper ground and packed by a single company in California [12-14]. An unopened bag of imported white peppercorns was found to contain the strain linked to the outbreak. The peppercorns were sold as "steam washed", which is primarily used to clean spices and not disinfect them. The imported product did have a Certificate of Analysis indicating that it was free of *Salmonella*. The processing facility was heavily contaminated with *Salmonella*, as approximately 40% of the environmental samples collected in the entire facility were *Salmonella* positive, and 100% of the environmental swabs from the grinding room were positive.

- 2009-2010, United States
  There was an outbreak of *Salmonella* Montevideo in the United States implicated the consumption of ready to eat dry sausage products that had been rolled in pepper after processing. Product testing found that both the black pepper and red pepper used to roll the dry sausage in were contaminated with *Salmonella* Montevideo [15, 16]. The black pepper was imported from Vietnam and the red pepper from both China and India. There was some indication that the black pepper had been treated with steam and the red pepper with ethylene oxide. Some of the black pepper shipments had certificates of analysis indicating that the product tested negative for *Salmonella*. In the aftermath of this outbreak, the USDA FSIS issued a directive to its' inspection staff, requiring them to verify that all ingredients added to a ready to eat meat or poultry product after the lethality treatment have met the food safety requirements for a ready to eat food [17].

2. Escherichia coli

While there have been reports of foodborne illness due to *Escherichia coli* contamination, these outbreaks do not occur nearly to the extent of *Salmonella*. One such outbreak occurred in Europe in 2011, where the Shiga-toxin producing *E. coli* O104:H4 was found on fenugreek seeds [18].

FDA compiled data on the prevalence of generic *Escherichia coli* on 50 g samples of spices post-treatment (Table 2) [6]. As not all species of *E. coli* are pathogenic, these values are not indicative of the pathogenic potential of these spice lots. However, the presence of *E. coli* on spice lots following

pathogen reduction treatment indicate that there has been a level of ineffectiveness in treatment or post-treatment controls meant to prevent contamination or additional growth.

Table 2. Frequency and prevalence of generic *E. coli* contamination in spice lots to which a pathogen reduction treatment had been applied (August 1, 2007-July 31, 2009)

Spice	N	# Positive	*E. coli* Lot Prevalence (%)	95% Confidence Level
All spices	25604	213	0.83	0.72-0.95
Spices subjected to different processes				
Raw/Whole spices	11119	30	0.27	0.18-0.39
Ground spices	9291	170	1.83	1.57-2.12
Whole black pepper	3742	18	0.48	0.28-0.76
Ground black pepper	3966	138	3.48	2.93-4.10
Spice treated with Ethylene Oxide	11601	4	0.03	0.01-0.09
Spices treated with steam	12086	208	1.72	1.50-1.97
Spice treated with irradiation	345	0	0.0	0.0-0.9
Spice treated with PPO	303	1	0.3	0.01-2
Spice treated with unspecified pathogen reduction process	1270	0	0.0	0.0-0.2

3. Bacillus cereus and *Clostridium perfringens*

Some, but not all, microbes produce spores which promote survival by preserving the DNA of the cells. Spores are significantly more resistant to environmental stressors than vegetative cells, and thus are important to monitor. Examples of sporeforming bacteria include *Bacillus cereus* and *Clostridium perfringens*.

Because *C. perfringens* or *B. cereus* may survive cooking processes, if foods containing these sporeformers are inadequately cooled after they are cooked, an opportunity may exist for rapid growth to a population that might cause illness. One study reported that meats and meat dishes seasoned with spices were the single most common cause of *B. cereus*-related foodborne illnesses in Hungary from 1960-1968 [19]. Further, *C. perfringens* has been isolated from a wide range of spices and herbs with counts typically less than 500 CFR/g [20]. However, these sporeformers require high numbers before causing illness (>10^5 CFU/g) and their related syndromes are relatively mild. This suggests that, though *B. cereus* and *C. perfringens* may be present in a food seasoned with spices, illnesses may be difficult to acquire. And when illness does occur, the symptoms may not be severe enough to warrant reporting or medical treatment. Therefore, the presence of these microorganisms in foods at low levels is not normally a health concern.

Despite their low risk as health concerns, the following outbreaks of *C. perfringens* and *B. cereus* in spices have been identified [21].

Table 3. Outbreaks of *C. perfringens* and *B. cereus* in spices [21].

Vehicle	Pathogen	Year	Country	Cases
Barbeque spice	*C. perfringens*	2011	Denmark	4

Red pepper spice	*C. perfringens*	2011	Denmark	37
Dried chilies	*C. perfringens*	2011	Denmark	3
Pepper	*C. perfringens*	2011	Denmark	10
Pepper	*C. perfringens*	2012	Denmark	9
Spice blend in couscous dish	*B. cereus*	2007	France	146
Curry	*B. cereus*	2009	Belgium	7
Rose-paprika	*B. cereus*	2009	Denmark	48
White pepper	*B. cereus*	2010	Denmark	112
Turmeric/curcuma	*B. cereus*	2011	Finland	4
Jeera Ground Cumin	*B. cereus*	2011	Finland	3
Turmeric/Curcuma	*B. cereus*	2011	Finland	19
Cinnamon	*B. cereus*	2011	Denmark	30
Pepper	*B. cereus*	2011	Denmark	52
Spices	*B. cereus*	2013	Finland	4

4. Clostridium botulinum

Although *C. botulinum* has been isolated from such dried spices as onion and garlic, outbreaks of botulism associated with spices are very rare. One outbreak was associated with garlic in oil in 1989 [22], while a more recent outbreak occurred in 2007, when eight cases of botulism were associated with the consumption of a hot dog chili sauce in Texas, Indiana, and Ohio [23]. While the viability of the sporeformers is not significantly affected by low water activity, germination is prevented. Therefore, the inability to germinate in a low water activity environment - even when anaerobic, such as an oil infusion - may account for the rarity of botulism outbreaks attributed to dried spices. Further, it is possible that dried spices may not be used as commonly as fresh spices in applications that support the growth of *C. botulinum*.

5. Other Pathogens

It is possible for other pathogens, such as *Listeria monocytogenes* and *Escherichia coli* O157:H7 [24-26], and although rare, parasites such as *Cyclospora cayetanensis,* to be occasionally found in fresh herbs [26]. *Listeria monocytogenes,* in particular, can contaminate foods and cause illness (listeriosis), which is largely associate with ready to eat (dairy products, deli meats) foods that have intrinsic characteristics (such as pH and water activity) that support the growth of *L. monocytogenes* [27]. Coagulase positive *Staphylococci* are typically not associated with spices [28, 29].

Antimicrobial Properties of Spices

The antimicrobial activity of spices has been studied for more than 100 years and a number of spices with antimicrobial properties have been identified [30-36]. Although some spices possess antimicrobial activity, they still can harbor large numbers of microorganisms including pathogens [7, 37]. While lower concentrations of microorganisms are found in spices with antimicobial properties, unfortunately the risk for contamination with pathogens is not sufficiently controlled by the antimicrobial properties of spices alone [38]. For example, although onion and garlic possess strong antimicrobial properties, *Salmonella* has been found in dehydrated garlic [7] and fresh onion have been implicated in a large *Salmonella* outbreak [39].

Typical aerobic bacterial populations range from less than 10^3 to over 10^7 CFU per gram [34, 40].

Degree of Antimicrobial Properties in Spices

It is well known that cinnamon, clove and mustard have strong antimicrobial activity [36]. In addition, onion, garlic and allspice possess strong antimicrobial properties, although *Salmonella* has been found in dehydrated garlic [7] and fresh onion has been implicated in a large *Salmonella* outbreak [39]. Spices having a lesser but significant antimicrobial effect include bay leaf, cumin, tarragon, thyme, rosemary, caraway, coriander and sage. Black pepper, ginger, and anise have little or no antimicrobial activity [41]. Antimicrobial activity has an impact on testing methodology, as the intrinsic antimicrobial properties of spices can interfere with microbiological assays intended to detect *Salmonella*. The following table summarizes antimicrobial activity for selected spices according to Billing and Sherman (1998). This study evaluated the degree of bacterial inhibition in spices based on an evaluation of available literature and online databases concerning foodborne bacteria that have been tested with spices.

Table 4. Antimicrobial Activity of Selected Spices and Herbs [41]

Inhibitory Effect	Selected Spice or Herb
Strong (>75% bacterial inhibition)	Bay Leaf, Cinnamon (Cassia), Clove, Cumin, Rosemary, Garlic, Onion, Allspice, Oregano, Tarragon, Thyme, Capsicums
Medium: (>50% to <75% bacterial inhibition):	Marjoram, Mustard, Caraway, Sage, Coriander, Dillweed, Nutmeg, Basil, Parsley
Weak or No Effect(<50% bacterial inhibition)	Black Pepper, Anise, Ginger, Parsley, White Pepper, Cardamom, Celery Seed

Antimicrobial Compounds in Spices

Many compounds with antimicrobial activity are naturally present in spices. The antimicrobial effect of spices has been related to compounds found in the essential oil fraction. These compounds include eugenol, carvacrol and thymol in cloves, cinnamon, sage and oregano; allicin in garlic; myristicin in

mace; and ally isothiocyanate in mustard. Low concentrations of oregano, however, were found stimulatory to lactic acid bacteria [36]. Furthermore, earlier work by [42] found that black pepper, allspice, and nutmeg stimulated growth and lactic acid production by a starter culture. The following table summarizes the essential oils known to have antimicrobial activity in spices.

Table 5. Essential Oils and Other Antimicrobial Constituents of Spices [43]

Compound	Found in	Reference
Allicin	Garlic	[44]
Allyl isothiocyanate	Mustard	[45]
Anethole	Anise, star anise, fennel	[46]
Carvacrol	Oregano, thyme	[45, 47]
Carvone	Caraway, dill	[47]
1,8-Cineole	Sage, rosemary, laurel, cardamom	[47]
Cinnamaldehyde	Cinnamon, cassia	[45, 48, 49]
p-Cymene	Cumin, thyme, oregano	[47]
Eugenol	Clove, allspice, cinnamon	[45-49]
Limonene	Celery seed, caraway, dill	[47]
Linalool	Coriander, sage, rosemary, basil	[47]
Thymol	Thyme, oregano	[46, 47, 50]
n-proply-allyl disulphides, di-*n*-propyl-disulphides	Onion	[51]

Impact of Antimicrobial Properties on Testing Methodology

Antimicrobial activity has an impact on testing methodology, as the intrinsic antimicrobial properties of spices can interfere with microbiological assays intended to detect *Salmonella*. Spices may require a neutralization step or a reduction in the sample to initial dilution ratio to enhance detection of *Salmonella*. The FDA has also recognized this and special procedures have been published in the Bacteriological Analytical Manual (BAM) for detection of *Salmonella* in selected spices [52]. A pre-enrichment in Trypticase Soy Broth (TSB) containing 0.5% potassium sulfite is recommended for onion and garlic, and the recommended procedure for allspice, cinnamon, cloves and oregano be diluted beyond their toxic levels. Allspice, cinnamon and oregano are diluted at 1:100 sample to broth (TSB) and

cloves are diluted 1: 1000 sample to broth (TSB) ratio. One study demonstrated that supplementing the non-selective enrichment broth with 2% corn oil resulted in a greater than 50% increase in the recovery of *Salmonella* from oregano [53]. These provisions further indicate the toxic effects that spices have on recovery of microorganisms, and in particular, *Salmonella*. No provisions for recovery of *Salmonella* have been published for those spices having weak or no known antimicrobial activity.

Prevalence of Bacteria in Raw Spices

Understanding the prevalence of microorganisms in raw spices can be useful information for spice processors when considering treatment methods to not only control pathogens, but also reduce spoilage bacteria. Aerobic plate count, yeast and mold, and other microbiological indicators are key quality measures.

A study sponsored by ASTA at the University of Wisconsin [54] showed that the following levels of microbial contamination of raw spices at import as reported by professional samplers (Table 6). The 108 spice samples analyzed originated from Indonesia, Brazil, Indian, Seychelles, Madagascar, France, Nigeria, Sierra Leone, Jamaica, Canada, United States, East Indies, West Indies, Greece, Mexico, and Spain. The study reports standard plate counts, bacterial spore counts, thermophilic anaerobe counts, proteolytic organisms and amylolytic organisms. No evidence of *Salmonella* or *E. coli* was detected in any of the samples.

Table 6. Microbial Profile of Raw Spices (microbial population ger gram (g) of spice).

Commodity	Aerobic Plate Count	Aerobic Mesophilic Sporeformer	Anaerobic Thermophilic Sporeformer	Yeast & Mold Population
Black pepper	$5.5x10^6$ to $5.0x10^7$	$5.5x10^6$ to $5.0x10^7$	$5.0x10^2$ to $3.0x10^5$	$1.0x10^1$ to $1.5x10^5$
Cassia	$1.0x10^3$ to $3.0x10^7$	$1.0x10^3$ to $5.5x10^6$	$1.0x10^1$ to $5.0x10^2$	$1.0x10^1$ to $3.5x10^5$
Celery seed	$7.5x10^4$ to $7.5x10^5$	$7.5x10^4$ to $7.5x10^5$	$1.0x10^1$ to $3.0x10^4$	0 to 9
Ginger	$5.5x10^3$ to $5.5x10^6$	$5.5x10^3$ to $5.5x10^6$	$1.0x10^1$ to $5.5x10^3$	$1.0x10^1$ to $3.0x10^4$
Mace	$5.5x10^3$ to $7.5x10^4$	$5.5x10^3$ to $7.5x10^4$	$1.0x10^1$ to $5.5x10^3$	0 to 9
Mustard seed	$1.0x10^3$ to $7.5x10^5$	$1.0x10^3$ to $3.0x10^4$	$1.0x10^1$ to $5.0x10^2$	0 to 9
Nutmeg	$1.0x10^3$ to $3.0x10^4$	$1.0x10^3$ to $5.5x10^3$	$1.0x10^1$ to $5.5x10^3$	$1.0x10^1$ to $5.0x10^2$
Oregano	$5.5x10^3$ to $1.5x10^5$	$1.0x10^3$ to $7.5x10^4$	$1.0x10^1$ to $5.5x10^3$	$1.0x10^1$ to $5.0x10^3$
Paprika (domestic)	$3.0x10^4$ to $5.5x10^6$	$5.5x10^3$ to $5.5x10^6$	$1.0x10^1$ to $5.5x10^3$	$1.0x10^1$ to $5.0x10^2$
Paprika (imported)	$5.5x10^6$ to $3.0x10^7$	$5.5x10^6$ to $3.0x10^7$	$1.0x10^1$ to $3.0x10^4$	$1.0x10^1$ to $5.0x10^2$
Rosemary	$5.5x10^2$ to $1.5x10^5$	$5.5x10^3$ to $3.0x10^4$	$1.0x10^1$ to $5.5x10^3$	$1.0x10^1$ to $3.0x10^4$

Since 1974, many studies have been conducted to evaluate the prevalence of bacteria in spices. However, many of these studies do not specify whether testing was conducted on raw or dried spices. Therefore, the extent of microbial reduction treatment prior to testing is unknown.

- 1982, South Africa
  A study of 36 difference spices showed the microbial contamination was >10^6 CFU/g in black pepper, coriander, pimento, paprika, and white pepper. *Salmonella* was found in paprika. Additionally, 4 CFU/g was found in white pepper and ginger, 7 CFU/g in ground pepper, and a coliform count of 2.0x10^6 CFU/g was noted in whole white pepper. Aflatoxin B1 and G1 were also detected in turmeric (12 µg/kg B1 and 8 µg/kg G1) and coriander (8 µg/kg B1 and 2 µg/kg G1) [28].

- 1982, USA
  A study of 10 different spices purchased at retail level found that the geometric means of the aerobic bacterial populations ranged from <100 to >10^8 per gram. However, for many of the spices more than 50% of the spices had microbial populations of <500,000 per gram. The exceptions were ginger and pepper, where 50% of the samples had populations <10^6 (ginger) and 10^7 (pepper) [40].

- 1985, Netherlands
  A study of 54 different retail spices showed that 43 of 54 spices were positive for *Clostridium perfringens*. Curry, paprika, white pepper, black pepper, ginger, basil, and curcuma were reported to have total aerobic plate counts of >10^7 CFU/g. Enterobacteriaceae were detected in oregano, tarragon, parsley, basic, and chervil at >10^5 CFU/g. *Bacillus cereus* was found in white pepper, ginger and mixed spices at >10^4 CFU/g [55].

- 1986, Japan
  In one study, the total aerobic plate count of dried black pepper, white pepper, turmeric, rosemary and basil were determined to be 3x10^3-5x10^7 per gram [56].

- 1986, Australia
  A study of 32 imported dried spices showed an aerobic plate count of 2.0x10^8 CFU/g in black pepper. *Clostridium perfringens* levels up to 1x10^3 CFU/g were detected in black and white peppercorns, broken mace, oregano, cinnamon, turmeric, and ginger. *Salmonella* was isolated from both black and white peppercorns [57].

- 1987, India
  A study of retail cumin seed and chili pepper showed that the aerobic plate counts were 1x10^8 CFU/g in cumin seed and 2x10^8 CFU/g in chili pepper. *Bacillus cereus* and *E. coli* were found in chili pepper [58].

- 1988, Nigeria
  A study of 230 dried, retail samples of red pepper, black pepper, thyme, and curry powder showed total bacterial counts up to 1.1x10^8 per gram and high loads of *Bacillus cereus* [59].

- 1989, USA
  A study of black pepper, white pepper, coriander seeds and fennel seeds showed aerobic plate counts of 10^7 CFU/g for black and white pepper and 10^5 CFU/g for coriander and fennel seeds.

Black pepper also test positive for *Salmonella* [60].

- 1991, USA
  A study of prepackaged spices in commercial containers was conducted. The study reported an aerobic plate count of $8.3x10^6$ CFU/g in black pepper and $6.9x10^6$ CFU/g in white pepper. Yeast and mold counts of $4.7x10^3$ CFU/g in black pepper and $9.5x10^4$ CFU/g in white pepper were also reported. (CITATION-can't find)

- 1991, Egypt
  An examination was conducted for aflatoxigenic molds in 130 samples of retail spices used in meat products. The most commonly isolated mold was *Aspergillus flavus*. Aflatoxin B_1 was detected in black and white pepper, while Aflatoxins B_1 and G_1 were detected in turmeric and coriander [61].

- 1995
  A comprehensive review of the microbiology of spices is published [37].

- 2003, India
  A study of 27 different spices offered for retail sale in India found that 51% had populations of total aerobic mesophilic bacteria which exceeded 10^6 per gram [62].

- 2006, Japan
  In an examination of 40 different spices imported into Japan and sold in retail shops, an incidence of *Salmonella* of 1.7% and 2.4% was reported in red and black pepper, respectively. Turmeric had the highest aerobic bacterial populations, with an average population of almost 10^7 per gram and the highest population of spore forming bacteria, with an average population of almost 10^5 per gram [63].

- 2006, USA
  A review was conducted on the recalls of spices in the United States between 1970 and 2003, and found that 20 of the 21 recalls were because of the presence of *Salmonella* [4].

- 2009, United Kingdom
  One study found that only 8.3% of dried spices and herbs sampled at production were unsatisfactory by EC or European Spice Association standards, while only 3.9% of dried spices and herbs sampled at retail were unsatisfactory by the same standards [38].

- 2013, USA
  The U.S. FDA sampled raw capsicum and sesame seed spices upon import and found the shipment prevalence for *Salmonella* was 3.3% for capsicum and 9.9% sesame seed [3].

- 2015, USA
  A study found that 19% of the spices sampled at retail had populations of aerobic-mesophilic

spore-forming bacteria greater than 10^5 per gram [64].

- 2017, USA
  The U.S. FDA sampled 11 spices at retail and found that no retail samples of cumin seed, sesame seed and white pepper were positive for *Salmonella*. A few samples of basil leaf, black pepper, coriander seed, curry powder, dehydrated garlic, oregano leaf, paprika, cayenne and red pepper tested positive for *Salmonella*, with the higher tested prevalence of 0.49% for dehydrated garlic, 0.56% for coriander and 0.64% for red pepper. These and other results were incorporated into the FDA's Draft Risk Profile on Pathogens and Filth in Spices [6].

- 2020, Ireland
  The Food Safety Authority of Ireland found that out of a total of 828 single samples of pre-packaged dried herbs and spices collected in 2017 and tested for *Bacillus cereus*, 22 samples (2.7%) were unsatisfactory (>10^5 per gram) while 79 samples (9.5%) were borderline (10^3 to 10^5 per gram), based on FSAI (FSAI, 2019) standards [65].

The high levels of microorganisms in spices reported by these studies further underscores the need to implement microbial reduction techniques in all aspects of the production, processing, packaging, and storage of spice products, not only to control pathogens as emphasized in other sections of this report, but also to prevent potential food spoilage.

Role of Microbiological Testing

In addition to GAPs, GMPs, and validated treatment methods, microbiological testing is frequently used as a critical component of spice companies' food safety and quality programs. Testing may be used to verify food safety programs and to meet customer specifications.

Test protocols for microbiological contamination must incorporate sound handling methods and statistically-guided sampling plans. Routine microbiological testing of products is used to determine the acceptance of purchased ingredients, raw materials, and finished products. Testing of spices for pathogens, including *Salmonella*, may be useful to screen for high rates of contamination entering a plant, but cannot completely eliminate risk. In instances where the contamination rate is low, the reliance on microbiological testing as the lone measure of food safety may be misleading as negative results do not always ensure safety [66]. For more information on the usefulness of pathogen testing, the International Commission for the Microbiological Safety of Foods has provided a decision tree [67].

Sample Collection and Preparation

Samples of product must be collected in order to perform microbiological testing. More stringent sampling plans are typically required for untreated products than for those that have undergone microbial reduction [68]. In fact, when spices are treated by validated methods, extensive testing post-treatment adds little to ensure food safety, increases cost, and may be unnecessary.

If sampling and testing is appropriate, the procedure for the collection of samples is key to ensuring accurate results. Collection instruments, such as scoops and bags, must be sterile to prevent cross-contamination. Proper hand washing techniques and the use of gloves is recommended. If product samples are collected prior to final processing and packaging, microbiological results may not be representative of product shipped due to cross-contamination in the processing or packaging equipment. Additional information regarding sample collection is available by FDA [69, 70].

A fundamental principle of lot acceptance sampling plans is that the samples collected will reflect the lot as a whole. For this reason, it is critical that the samples be collected at various points throughout the entire lot. This is particularly important where the microbial population is not homogenous, such as imported raw spices that may be comprised of many batches or sub-lots. For non-homogenous lots, an increased number of samples may be required to properly evaluate the lot.

An analytical unit is the aliquot of a sample that is actually tested. A typical analytical unit is 25 to 375 grams per sample, although this may be increased if desired. When testing for indicator microorganisms, such as aerobic or coliform bacteria, compositing multiple samples is a common technique to decrease the cost of testing. The disadvantage of compositing samples is that an individual sample with high levels of contamination may be diluted by mixing with samples with much lower or no contamination.

When more than one sample is analyzed for a microbiological attribute, a two- or three-class sampling plan may be applied to evaluate results. The attributes of these sampling plans are given below:

n = number of analytical samples

c = number of samples that may be tolerated in the marginally acceptable range (area between m and M)

m = value below which all values are acceptable

M = value at which all values above are defective

Two-Class Sampling Plans

A two-class sampling plan is appropriate when zero positives are permitted (e.g. testing for *Salmonella*). In a two-class sampling plan, c = 0 and m = M in that there is no marginal range of acceptance and no sample may contain levels greater than m.

For *Salmonella* analysis, no greater than fifteen (15) analytical units (25 grams each) may be composited into one 375-gram test sample and tested in its entirety. When compositing is performed, it is important to ensure that equal amounts of each sample be included in the composite. Pulling a 375-gram sample from a bag of unequally mixed portions of analytical units is not acceptable. For spices imported into the United States and submitted for approval for reconditioning due to the detection of Salmonella, the FDA defines the sampling plan [70].

Three-Class Sampling Plans

A three-class sampling plan may be appropriate when a proportion of sample units may yield test values in a marginally acceptable range without causing consequent problems [67]. This is often true for testing indicator microorganisms, such as aerobic bacteria, coliforms, yeast, and mold. It may also be

appropriate for certain bacterial pathogens where a tolerance can be established in a product without jeopardizing safety, such as *C. perfringens, B. cereus,* or *S. aureus*. In a three-class sampling plan with n = 5, c = 1, m = 1,000, and M = 10,000, one out of five samples may test above 1,000 but lower than 10,000 with the lot still being considered acceptable.

To further assist in the development of a three-class sampling plan, a statistician should be consulted in order to select appropriate values for m and M. Additional information on sampling plans is available from ICMSF [67] and the FDA.

Statistical Basis of Lot Acceptance Sampling Plans

Factors that influence the effectiveness of a sampling plan include whether random samples can be collected from a lot, how samples are prepared, and the sensitivity and specificity of the analytical method [67]. Lot acceptance sampling plans assume the microbial population to be randomly distributed throughout the lot. This is often not true, especially for foods that are not liquids. For this reason, better information about the true microbiological population within a lot can be obtained by analyzing more than one sample. The number of samples that are collected from a lot is a balance between risk, accuracy, available resources, time, and cost.

Table 2 demonstrates the relation of number of samples collected to the contamination (defect) rate. Table 2 indicates that:

- If 10 samples are collected across a lot that has *Salmonella* in 1% of samples, there is a 90% probability that *Salmonella* will be not detected and the lot will be accepted.
- If 10 samples are collected across a lot that has *Salmonella* in 10% of samples, there is a 35% probability that *Salmonella* will be not detected and the lot will be accepted.
- If 15 samples are collected across a lot that has *Salmonella* in 5% of samples, there is a 46% probability that *Salmonella* will be not detected and the lot will be accepted.

Table 7. Probability of acceptance (Pa) of defective product using a two-class sampling plan

PERCENT DEFECTS	NUMBER OF SAMPLES						
	1	5	10	15	20	30	60
1	0.99	0.95	0.90	0.86	0.82	0.74	0.55
2	0.98	0.90	0.82	0.74	0.67	0.55	0.30
5	0.95	0.77	0.60	0.46	0.36	0.21	0.05
10	0.90	0.59	0.35	0.21	0.12	0.04	<0.005
20	0.80	0.33	0.11	0.04	0.01	<0.005	<0.005

For the spice industry, the percent defective (in this case, *Salmonella* positive) is less than 1% (see Table 7). This means that even with a large number of samples, the probability of accepting a *Salmonella*-positive lot is high. However, sampling and testing is still an important part of an overall food safety

program, and conclusions may be drawn even with a relatively small number of samples [71].

Legal and Regulatory Requirements

The United States Congress passed the Food Safety Modernization Act (FSMA) in 2010, which was signed into law by President Obama in 2011. FSMA requires food companies that operate in or import to the United States to have a food safety plan and comply with additional requirements intended to ensure food is safe to eat.

The FDA's Preventive Controls Rule for Human Foods outlines the requirements for companies that import, process or sell food in the United States must have in place as a part of their food safety plans. Food safety plans must be overseen by a Preventive Controls Qualified Individual. Companies must conduct a hazard analysis that includes an assessment of microbiological hazards. FDA's Appendix I of the Hazard Analysis and Risk-Based Preventive Controls for Human Food: Draft Guidance for Industry includes an overview of hazards that are associated with specific food products, including spices and seasonings [72].

Identified hazards must have validated process controls in place that provide scientific justification that the control is effective at mitigating the hazard. This control may be applied by a customer or downstream user in the supply chain. However, if this is the case, then the product must be labeled that it has not been processed to control the hazard (e.g. "Not Processed to Control Salmonella"). Conversely, if a supplier has applied the control, then the company must implement supply chain controls, such as audits and review of food safety documentation to ensure the supplier has adequately controlled the hazard.

The successfulness of a company's food safety plan and preventive controls can be verified through different actions. For example, environmental monitoring can provide insight into a facility's microbiological profile, as well as verification of the effectiveness of a facility's overall sanitation and microbial control programs. FDA expects that food facilities will engage in environmental monitoring for Ready-to-Eat (RTE) foods that are exposed to the environment post-lethality. Another verification activity is product testing, which can be used to verify that a process validation for microbiological hazards was effective. While verification activities are an important part of a preventive controls food safety plan, they are not sufficient on their own as a preventive control, but rather provide verification of a preventive controls successfulness.

It is noteworthy that the FDA considers spices sold to customers to be RTE products by nature according to the 2017 Food Code [73]. The general public might not consider spices to be a food safety risk and frequently uses them without subsequent cooking. Although most foods made by food manufacturers are cooked or processed prior to consumption, many are also consumed without the benefit of a lethality step, such as cooking at the appropriate temperature and time by boiling, baking, etc [73]. The implication of this is that companies must ensure that spice products are processed to control potential hazards prior to being sold to consumers.

The Foreign Supplier Verification Program requires importers of foods into the United States to ensure that any imported food complies with U.S. requirements. Importers must have documented FSVPs that demonstrate the hazards and supply chain controls that are in place.

ASTA has developed additional resources for the spice industry to support compliance with these requirements, including compliance information available on the ASTA website, recorded webinars, and research and resources on validation.

Conclusions & Additional Resources on Best Practices for Food Safety

Although it is clear that raw spices may be contaminated with microbial pathogens, there are treatment and testing options available to ensure food safety and meet quality criteria. Comprehensive food safety plans must include good agricultural practices, supply chain controls, good manufacturing practices, and validated treatment processes to control pathogens [1]. Information is available regarding the typical microbiological profiles of untreated and treated spices and regarding the antimicrobial properties of certain spices. All of this should be taken into account to design a sound sampling and testing regime to help minimize food safety risk. Furthermore, spice companies must comply with all legal and regulatory requirements that are designed to prevent foodborne illness.

ASTA has developed a number of resources on Good Agricultural Practices (GAPs) [74], Good Manufacturing Practices (GMPs) [75], including pest management, and HACCP Plans [76]. HACCP is the acronym for Hazard Analysis Critical Control Point and ASTA has developed a HACCP Guide to address critical food safety issues specific to the spice industry [77]. In addition to the pre-requisite programs, companies are enhancing their quality and food safety systems to incorporate global food safety programs such as those defined by the Global Food Safety Initiative [78]. All of the food safety programs recognized by the Global Food Safety Initiative require HACCP programs.

Furthermore, ASTA has compiled information on the identification and prevention of adulteration [79], cleanliness as it pertains to the U.S. FDA's Defect Action Levels [80], and environmental monitoring [81]. Lastly, guidance has been published for the detection of *Salmonella* in seasoning, herbs, and spice matrices, as well as laboratory best practices [82].

References

1. American Spice Trade Association (ASTA),. *Clean, Safe Spices White Paper. Guidance from the American Spice Trade Association.* 2017. *https://s03a5eeb214d1b536.jimcontent.com/download/version/1458129972/module/8126297595/name/ASTAGuidance.pdf.*
2. Beuchat, L.R., et al., *Low-water activity foods: increased concern as vehicles of foodborne pathogens.* J Food Prot, 2013. **76**(1): p. 150-72.
3. Van Doren, J.M., et al., *Foodborne illness outbreaks from microbial contaminants in spices, 1973-2010.* Food Microbiology, 2013. **36**(456-464).

4. Vij, V., et al., *Recalls of Spices Due to Bacterial Contamination Monitored by the U.S. Food and Drug Administration: The Predominace of Salmonella.* J. Food. Prot., 2006. **69**: p. 233-237.
5. Zweifel, C. and R. Stephan, *Spices and herbs as source of Salmonella-related foodborne diseases.* Food research International, 2012. **45**: p. 765-769.
6. U.S.FDA, *Risk Profile: Pathogens and Filth in Spices.* 2017. *https://www.fda.gov/media/108126/download*.
7. Zhang, G., et al., *Prevalence of Salmonella in 11 Spices Offered for Sale from Retail Establishments and in Imported Shipments Offered for Entry to the United States.* J. Food Protection, 2017. **80**(11): p. 1791-1805.
8. Laidly, R., S. Handzel, and D. Severs, *Salmonella weltevreden outbreak associated with contaminated pepper.* Epidemio. Bull., 1974. **18**: p. 80.
9. Gustavsen, S. and O. Breenk, *Investigation of an outbreak of Salmonella oranienburg infections in Norway, caused by contaminated black pepper.* Am. J. Epidemiol, 1984. **119**: p. 806-812.
10. Lehmacher, A., J. Beckemuhl, and S. Aleksic, *Nationwide outbreak of human salmonellosis in Germany due to contaminated paprike and paprika-powedered potato ships.* Epidemiol. Infect., 1995. **115**: p. 501-511.
11. Sotir, M.J.G., et al., *Outbreak of Salmonella Wandsworth and Typhimurium infections of infants and toddlers traced to a commercial vegetable-coated snack food.* Pediatr. Infect. Dis. J., 2009. **28**(12): p. 1041-1046.
12. California Department of Public Health, Food and Drug Branch., Emergency Response Unit (CDPH/FDB/ERU), *Investigation of Union International Food Company Salmonella Rissen Outbreak Associated with White Paper.* 2010. *Available at: http://www.cdph.ca.gov/pubsforms/Documents/fdbEIRUFIC2009.pdf.*
13. Hajmeer, M. and C. Myers, *Outbreak of Salmonella Rissen Associated with Ground White Pepper -- The Environmental Investigation.* , in *International Association for Food Protection 2010 Annual Conference*. 2011: Anaheim, CA.
14. Higa, J. *Outbreak of Salmonella Rissen Associated with Ground White Pepper: The Epi Investigation*. in *Union International Investigation Meeting Presentation, Quarterly Scientific Seminar, California Department of Public Health.* 2011. *Available at: http://www.cdph.ca.gov/programs/DFDRS/Documents/QSS_Presentation_SRissen_and_%20white%20pepper_010611.pdf.*
15. Centers for Disease Control and Prevention (CDC)., *Salmonella Montevideo infections associated with salami products made with contaminated imported black and red pepper -- United States, July 2009-April 2010.* MMWR, 2010. **59**(50): p. 1647-1650.
16. Gieraltowski, L., et al., *Nationwide outbreak of Salmonella Montevideo infections associated with contaminated imported black and red pepper: Warehouse membership cards provide critical clues to identify the source.* Epidem. Inf., 2012. **1**(1): p. 1-9.
17. USDA FSIS, *Instructions for verifying all steps in the processing of ready-to-eay meat and poultry products. FSIS Notice 01-11.* 2011.
18. European Food Safety Authority (EFSA)., *Scientific Opinion on the risk posed by Shiga Toxin-producing E. coli (STEC) and other pathogenic bacteria in seeds and sprouted seeds.* EFSA Journal 2011, 2011. **9**(11): p. 2424.
19. Goepfert, J.M., W.M. Spira, and H.U. Kim, *Bacillus cereus: food poisoning organism. A review.* J. Milk Food Technol., 1972. **35**: p. 213-227.
20. DeBoer, E. and E.M. Boot, *Comparison of methods for isolation and confirmation of Clostridium perfringens from spices and herbs.* J. Food Protection, 1983. **46**(533-536).
21. Ontario Agency for Health Protection and Promotion (Public Health Ontario)., *Case Study: Pathogens and Spices*, N. Parto, Editor. 2016, Queen's Printer for Ontario: Toronto, ON.

22. Morse, D.L., et al., *Garlic-in-Oil Associated Botulism: Episode Leads to Product Modification.* American Journal of Public Health, 1990. **80**: p. 1372-1373.
23. Juliao, P.C., et al., *National Outbreak of Type A Foodborne Botulism Associated With a Widely Distributed Commercially Canned Hot Dog Chili Sauce* Clinical Infectious Diseases, 2016. **56**(3): p. 376-382.
24. Horr, T. and A.K. Pradhan, *Evaluation of public health risk for Escherichia coli O157:H7 in cilantro.* Food Research International, 2020. **136**: p. 109545.
25. Kara, R., et al., *Microbiological Quality of Salmonella spp./Listeria monocytogenes of Spices in Turkey.* Research Journal of Microbiology, 2015. **10**(9): p. 440-446.
26. U.S.FDA, *FDA Sampling Fresh Herbs, Guacamole and Processed Avocado.* 2020. *https://www.fda.gov/food/cfsan-constituent-updates/fda-sampling-fresh-herbs-guacamole-and-processed-avocado*.
27. American Spice Trade Association (ASTA), *FAQs for the American Spice Trade Association (ASTA) on the Potential Risks Related to Listeria monocytogenes in Spices.* 2019. *https://www.astaspice.org/download/14781*.
28. Baxter, R. and W.H. Holzapfel, *A microbial investigation of selected spices, herbs, and additives in South Africa.* J. Food Science, 1982. **47**: p. 570-578.
29. Powers, E.M., R. Lawyer, and Y. Masuoka, *Microbiology of processed spices.* J. Milk Food Technol., 1975. **38**(683-87).
30. Beuchat, L.R., *Antimicrobial properties of spices and their essential oils*, in *Natural Antimicrobial Systems and Food Preservation*, V.M.B. Dillon, A.G., Editor. 1994, CAB International: Wallingford (UK). p. 167-179.
31. Beuchat, L.R. and D.A. Golden, *Antimicrobials occuring naturally in foods.* Food Technology, 1989. **43**: p. 134-142.
32. Hammer, K.A., C.F. Carson, and T.V. Riley, *Antimicrobial activity of essential oils and other plant extracts.* J. Applied Microbiology, 1999. **86**(985-990).
33. Nakatani, N., *Antioxidative and antimicrobial constituents of herbs and spices*, in *Spices, Herbs, and Edible Fungi*, G. Charalambous, Editor. 1994, Elsevier: Amsterdam. p. 251-272.
34. Shelef, L.A., O.A. Naglik, and D.W. Bogen, *Sensitivity of some common food-borne bacteria to the spices sage, rosemary, and allspice.* J. Food. Sci., 1980. **45**(1042-1044).
35. Snyder, O., *Antimicrobial Effects of Spices and Herbs*. 1997, Hospitality Institute of Technology and Management: St. Paul, MN.
36. Zaika, L.L. and J.C. Kissinger, *Inhibitory and stimulatory effects of oregano on Lactobacillus plantarum and Pediococcus cerecisiae.* J. Food Sci, 1981. **46**: p. 1205-1210.
37. McKee, L.H., *Microbial Contamination of Spices and Herbs: A Review.* Lebensm-Wiss, U.-Technol., 1995. **28**: p. 1-11.
38. Sagoo, S.K., et al., *Assessment of the microbiological safety of dried spices and herbs from production and retail premises in the United Kingdom.* Food Microbiology, 2009. **26**: p. 39-43.
39. Center for Disease Control and Prevention (CDC)., *Outbreak of Salmonella Newport Infections Linked to Onions. https://www.cdc.gov/salmonella/newport-07-20/index.html* 2020.
40. Schwab, A.H., et al., *Microbiological quality of some spices and herbs in retail markets.* Appl. Environ. Microbiol., 1982. **44**: p. 627-630.
41. Billing, J. and P.W. Sherman, *Antimicrobial functions of spices: Why some like it hot.* The Quarterly Review of Biology, 1998. **73**: p. 3-49.
42. Kissinger, J.C. and L.L. Zaika, *Effect of major spices in Lebanon bologna on acid production by starter culture organisms.* J. Food Prot., 1978. **41**: p. 429-431.
43. Zaika, L.L., *Spices and Herbs: Their antimicrobial activity and its determination.* J. Food Safety, 1988. **9**: p. 97-118.

44. Cavillito, C.J. and J.H. Bailey, *Allicin, the antibacterial principle of Allium sativum. I. Isolation, physical properties and antibacterial action.* J. Am. Chem. Soc., 1944. **66**: p. 1950-1951.
45. Blum, H.B. and F.W. Fabian, *Spice oils and their components for controlling microbial surface growth.* Fruit Projects J., 1943. **22**: p. 326-329, 347.
46. Hitokoto, H., et al., *Inhibitory effects of spices on growth and toxin production of toxigenic fungi.* Appl. Environ. Microbiol., 1980. **39**: p. 818-822.
47. Kellner, W. and W. Kober, *Möglichkeiten der Verwendung ätherischer Öle zur Raumdesinfektion. 2. Ermittlung der für die keimtötende Wirkung verantwortlichen Einzelbestandteile relativ unschädlicher ätherischer.* Öle. Arzneim. Forsch, 1955. **5**: p. 224-229.
48. Bullerman, L.B., F.Y. Lieu, and S.A. Seier, *Inhibition of growth and aflatoxin production by cinnamon and clove oils, cinnamic aldehyde and eugenol.* J. Food Sci., 1977. **42**: p. 1107-1109, 1116.
49. Ueda, S., H. Yamashita, and Y. Kuwabara, *Inhibition of Clostridium botulinum and Bacillus sp. by spices and flavoring compounds.* Nippon Shukuhin Kogyo Gakkaishi, 1982. **29**: p. 389-392.
50. Buchanan, R.L. and A.J. Shepard, *Inhibition of Aspergiluuus parasiticus by thymol.* J. Food Science, 1981. **46**: p. 976-977.
51. Hargreaves, L.L., et al., *The Antimicrobial Effects of Spices, Herbs and Extracts from these and Other Food Plants*, in *Scientific & Technical Surveys. No. 88*. 1975, Leatherhead Food RA: Surrey, England.
52. U.S.FDA, *Bacteriological Analytical Manual (BAM) Chapter 5: Salmonella. Part C. Preparation of foods for isolation of Salmonella subpart 10. Spices.*2020. *Available at: https://s27415.pcdn.co/wp-content/uploads/2020/01/64ER20-7/Microbial/1-Bacteriological-Analytical-Manual-BAM_Ch5_-Salmonella-_-FDA.pdf*.
53. Beaubrun, J.J.-G., et al., *Evaluation of corn oil as an additive in the pre-enrichment step to increase recovery of Salmonella enterica from oregano.* Food Microbiology, 2016. **57**: p. 195-203.
54. Julseth, R.M. and R.H. Deibel, *Microbial Profile of Selected Spices and Herbs at Import.* J. of Milk and Food Technol., 1974. **37**: p. 414-419.
55. de Boer, E., W.M. Spiegelenberg, and F.W. Janseen, *Microbiology of spices and herbs.* Antonie van Leeuwenhoek, 1985. **51**: p. 435-438.
56. Juri, M.L., et al., *Distribution of Microorganisms in Spices and Their Decontamination by Gamma-irradiation.* Agricultural and Biological Chemistry, 1986. **50** (2): p. 347-355.
57. Pafumi, J., *Assessment of the Microbiological Quality of Spices and Herbs.* J Food Protection, 1986. **49**(12): p. 958-963.
58. Bhat, R., H. Greeta, and P.R. Kulkarnia, *Microbial Profile of Cumin Seeds and Chili Powder Sold in Retail Shops in the City of Bombay.* J Food Protection, 1987. **50**(5): p. 418-419.
59. Antal, S.P., *Study of the Bacillus fora of Nigerian spices.* International Journal of Food Microbiology, 1988. **6**: p. 259-261.
60. Satchell, F.B., et al., *Microbiological Survey of Selected Imported Spices and Associted Fecal Pellet Specimens.* Journal of Association of Official Analytical Chemists, 1989. **72**(4): p. 632-637.
61. Aziz, N.H. and Y.A. Youssef, *Occurrence of aflatoxins and aflatoxin-producing moulds in fresh and processsed meat in Egypt.* Food Additive and Contaminants, 1991. **8**(3): p. 321-331.
62. Banerjee, M. and P.K. Sarkar, *Microbiological quality of some retail spices in India.* Food Research International, 2003. **36**: p. 469-474.
63. Hara-Kudo, Y., et al., *Salmonella Prevalence and Total Microbial and Spore Populations in Spices Imported to Japan.* Journal of Food Protection, 2006. **69**(10): p. 2519-2523.
64. Hariram, U. and R. Labbe, *Spore Prevalence and Toxigenicity of Bacillus cereus and Bacillus thuringiensis Isolates from U.S. Retail Spices.* Journal of Food Protection, 2015. **78**(3): p. 590-596.

65. FSAI, *Survey of the microbiological safety of pre-packaged dried herbs and spices.* 2020.
66. *IFT Expert Report on Emerging Microbiological Food Safety Issues, Implications for Control in the 21st Century. http://citeseerx.ist.psu.edu/viewdoc/download;jsessionid=E075268A64809FE88892ACAFB3381C70?doi=10.1.1.410.2714&rep=rep1&type=pdf*
67. International Commission for the Microbiological Safety of Foods. *Microorganisms in Foods 7, Microbiological Testing in Food Safety Management*. 2002, Kluwer Academic Plenum Pubishers: New York, NY.
68. U.S.FDA, *BAM Chapter 1: Food Sampling/Preparation of Sample Homogenate.* 2003. *https://www.fda.gov/food/laboratory-methods-food/bam-chapter-1-food-samplingpreparation-sample-homogenate.*
69. FDA Investigations Operations Manual. *4.3 Collection Technique.* 2020. *https://www.fda.gov/media/80360/download*
70. FDA Investigations Operations Manual *4.5 Sampling: Preparation, Handling, Shipping, Sample Schedule 1, Salmonella Sampling*. 2020. *Available at: https://www.fda.gov/media/80360/download*
71. Hoelzer, K. and R. Pouillot, *Practical considerations for the interpretation of microbial testing results based on small numbers of samples.* Foodborne Pathogens and Disease, 2013. **10**(11): p. 907-915.
72. U.S. FDA., *Draft Guidance for Industry: Hazard Analysis and Risk-Based Preventive Controls for Human Food. FDA-2016-D-2343*. 2018, Center for Food Safety and Applied Nutrition.
73. U.S.FDA, *2017 Food Code. https://www.fda.gov/food/fda-food-code/food-code-2017.* 2017.
74. American Spice Trade Association (ASTA), *General Guidelines for Good Agricultural Practices for Spices.* 2020. Available at: *https://www.astaspice.org/download/10430.*
75. American Spice Trade Association (ASTA), *Good Manufacturing Practice (GMP) Guide for Spices.* 2015. *Available at: https://www.astaspice.org/food-safety-technical-guidance/best-practices-and-guidance/good-manufacturing-practice-gmp-guidelines-for-spices/.*
76. *USDA Prerequisite Program. http://www.fsis.usda.gov/PDF/Training_Prerequisite_Programs.pdf*
77. American Spice Trade Association (ASTA)., *HACCP Guide for Spices and Seasonings*. 2015, https://www.astaspice.org/food-safety-technical-guidance/best-practices-and-guidance/haccp-guide-to-spices-and-seasonings/.
78. *The Global Food Safety Initiative. https://mygfsi.com/*.
79. American Spice Trade Association (ASTA), *Identification and Prevention of Adulteration.* 2016. *Available at: https://www.astaspice.org/food-safety-technical-guidance/best-practices-and-guidance/identification-prevention-adulteration-guidance-document/.*
80. American Spice Trade Association (ASTA), *ASTA Cleanliness Specifications for Spices, Seeds, and Herbs (Foreign and Domestically Produced).* 2014. *Available at: https://www.astaspice.org/food-safety-technical-guidance/best-practices-and-guidance/cleanliness-specifications/.*
81. American Spice Trade Association (ASTA), *ASTA Guidance on Environmental Monitoring Programs.* 2019. *Available at: https://www.astaspice.org/food-safety-technical-guidance/best-practices-and-guidance/enviromental-monitoring/.*
82. American Spice Trade Association (ASTA), *Salmonella Detection Methods and Laboratory Best Practices for Seasonings, Herbs, and Spice Matrices: A White Paper*, J.S. Dickson, Editor. 2020, Microbiology Task Force.