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## Microbiology of Processed Spices

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### ABSTRACT

The microbiology of spices procured by the Army, Navy, Marines and Air Force was determined. The incidence of *Clostridium perfringens* in the spices analyzed was 15%. The organism was found in 4 out of 7 types of spice and in 53% of the oregano samples. No other bacteria of public health significance were found. The microflora of spices from 10 different brands varied widely.

Some spices harbor great numbers of microorganisms (3) including sporeforming bacteria and spoilage organisms (7). Unless spices are treated they may contaminate menu items and cause spoilage and may even introduce harmful organisms (11). While the bacteriological safety of spices can effectively be achieved by several fumigation practices (ethylene oxide, propylene oxide, microwave and gamma irradiation), the methods are limited by cost, time required to completely destroy microorganisms and their effect on flavor and color of some spices (12). Furthermore, the amount of residual ethylene oxide and propylene oxide in spices is limited by the Food and Drug Administration (FDA). Although gamma irradiation was found to be more effective than ethylene oxide in reducing the bacterial population of spices (12), irradiation of spices is not permitted by the FDA.

Because of the aforementioned limitations spices are produced with various microbial loads. Although a few studies have been done to determine the microflora of black and red pepper (3), some untreated spices (8) and selected spices and herbs at import (7) there is still little information regarding the microbiology of processed spices commonly used by the ordinary consumer including the military forces.

The purpose of this investigation was to determine if the military is receiving satisfactory products and whether or not a potential health problem is associated with spices procured by the military.

### MATERIALS AND METHODS

#### Number and source of samples

A total of 114 samples of seven types of spices and herbs were received from 16 military bases. Army, Navy, Marine, and Air Force bases located in different geographical areas of the United States were represented. Spices were procured locally by each base and represented 10 different processors. With the exception of bay leaves all spices were ground or powdered. Upon receipt all samples were stored in their sealed containers at 23 C until analyzed.

#### Preparation of sample

Ten grams of each spice, including herbs, were weighed into a sterile, tared blender jar, and blended for 1 to 2 min in 90 ml of sterile phosphate buffered water (SBW) (1, 2). Dilutions were made from the initial blend (1:10) by transferring 10 ml into 90 ml SBW (2, 6). The lowest dilution cultured was 1:100 to avoid possible inhibition of growth by bactericidal or bacteriostatic properties of some spices (5, 8). For *Clostridium perfringens* counts, this dilution was achieved by spreading 0.1 ml of 1:10 dilution on duplicate plates of Shahidi Ferguson Perfringens (SFP) agar.

#### Inhibition of bacterial growth by spices

To determine if the spices tested would inhibit bacterial growth, *Escherichia coli* and *Staphylococcus aureus* were inoculated into tubes of Lauryl Sulfate Tryptose (LST) broth and Trypticase Soy Broth (TSB) containing 10% sodium chloride (NaCl), respectively, to which was added 1 ml of 1:10 dilution of each spice. Excellent growth of both organisms was achieved in their respective media in less than 24 h at 35 C, indicating that the spices were not inhibitory at the concentration tested. Subsequent recovery of both organisms by Standard Methods described below also was achieved. To allow a margin of safety the lowest "in-use" dilution of spices for all counting procedures was 1:100.

#### Media

All media were purchased from Difco Laboratories, Detroit, Michigan.

#### Aerobic plate count

One milliliter of dilutions ranging from  $10^{-2}$  to  $10^{-5}$  was pipetted into duplicate petri plates, poured with Plate Count Agar and incubated at 35 C for 48 h.

#### Yeast and mold count

Dilutions prepared for aerobic plate counts also were used for making yeast and mold counts. One milliliter of each dilution was added to petri plates and the plates were poured with Potato Dextrose agar (PDA), acidified to pH 3.5. Plates were incubated at 23 C for 5 days before counting.

#### Coliform and fecal coliform count

Coliforms were estimated by a 3-tube most probable number (MPN) determination in Lauryl Sulfate Tryptose (LST) broth by Standard Methods (2). Gas producing LST tubes were confirmed by use of Brilliant Green Lactose Bile (2%) broth (BGLB). Fecal coliforms were estimated by transferring growth from positive LST tubes to EC broth and incubating at 45.5 C for 48 h (2). Counts determined from MPN tables are presented as <30/g when all tubes at the 1:100 dilution are negative.

#### Coagulase positive staphylococci

Coagulase positive staphylococci were estimated by a modification of the 3-tube MPN procedure, using the same dilutions as for the coliform count. To allow for recovery of injured cells, 1 ml of appropriate dilutions was added to 5 ml of TSB and incubated at 35 C for 3 h. An equal volume of TSB containing 19.5% NaCl was then added to each tube to yield a final salt concentration of 10% (6, 9). After incubation at 35 C for 48 h, 0.1 ml of the TSB-NaCl culture was spread on each of

TABLE 1. Microbiology of processed spices used by the Armed Forces

Spice	No. of samples	Range of counts/g of spice					
		APC	Yeast and molds	Coliforms (MPN)	Fecal coliforms	Coagulase positive staphylococci (MPN)	<i>Clostridium perfringens</i>
Bay leaves	16	<100 to 30,000	<100 to 670,000	<30	<30	<30	<100 to 500
Cayenne pepper	15	3400 to 9,100,000	<100 to 20,000	<30 to 2,400	<30	<30	<100 to 250
Chili powder	18	100 to 540,000	<100 to 8,100	<30	<30	<30	<100
Cinnamon	18	10,700 to 780,000	<100 to 4,600	<30 to 230	<30	<30	50 to 300
Garlic powder	16	3700 to 980,000	<100 to 500	<30 to 230	<30	<30	<100
Mustard powder	16	500 to 5300	<100 to 2,200	<30	<30	<30	<100
Oregano	15	1200 to 3,600,000	<100 to 27,000	<30	<30	<30 to 30	50 to 2850

two plates of Vogel Johnson Agar. Plates were incubated at 35 C and examined after 24 and 48 h for the presence of smooth black colonies with yellow zones. Two or more typical representative colonies were transferred to tubes of Brain Heart Infusion (BHI) broth and tested for coagulase production according to standard procedures (2). Counts, determined from MPN tables, are presented as <30/g when all tubes at the 1:100 dilution were negative.

#### *Clostridium perfringens*

*Clostridium perfringens* was counted by spreading 0.1 ml of 1:10 and 1:100 dilutions onto duplicate plates of Shahidi-Ferguson Perfringens (SFP) agar (10). Plates were overlaid with 10 ml of SFP overlay agar (egg yolk omitted), placed into gaspak anaerobic jars (BBL) and incubated at 35 C for 24 h. Black colonies surrounded by a zone of precipitate were counted and confirmed in Lactose Motility (LM) agar. Lactose Motility agar was steamed for 10 min and cooled immediately before use. Inoculated tubes were incubated at 35 C for 24 h. Non-motile, lactose positive cultures in LM agar, exhibiting typical microscopic morphology, were considered to be *C. perfringens* (10). Counts are presented as 50/g when one colony was confirmed from 0.1 ml of a 1:10 dilution. When no colonies were observed, counts are presented as <100/g since the lowest dilution per gram was 1:100.

## RESULTS

### Bay leaves

Sixteen samples of bay leaves were analyzed. The APC range from <100/g to 30,000/g (Table 1). Yeast and mold counts were less than 10,000/g (Table 2) except for one sample of brand 9, which had a count of 670,000/g (Tables 1 and 3). *C. perfringens* (Tables 3 and 4) was found in two (12%) of the samples of bay leaves, with counts of 300/g and 500/g. Both were from brand 3 (Table 5). No coliforms, fecal coliforms, or coagulase positive staphylococci were found at the lowest dilutions tested and are reported as <30/g (Tables 1, 4, and 6).

### Cayenne pepper

Fifteen samples of ground cayenne pepper were analyzed. The APC ranged from 3400/g to 9,100,000/g (Table 1). Thirteen samples had APC's greater than 100,000/g and nine had counts greater than 1 million/g

(Table 7). Yeast and mold counts ranged from <100/g to 20,000/g (Tables 1 and 2).

TABLE 2. Yeast and mold count of processed spices used by the Armed Forces

Spice	Total No. of samples	Number of samples containing (per gram)				
		<100	101 to 1000	1001 to 10,000	10,001 to 30,000	>30,000
Bay leaves	16	7	3	5	0	1
Cayenne pepper	15	8	2	3	2	0
Chili powder	18	17	0	1	0	0
Cinnamon	18	3	9	6	0	0
Garlic powder	16	11	5	0	0	0
Mustard powder	16	12	3	1	0	0
Oregano	15	2	3	8	2	0
TOTAL	114	60	25	24	4	1

TABLE 3. *Clostridium perfringens* count in processed spices used by the Armed Forces

Spices	No. of samples	Number of samples containing (per gram)			
		<100	100 to 500	501 to 1000	1001 to 3000
Bay leaves	16	14	2	0	0
Cayenne pepper	15	13	2	0	0
Chili powder	18	18	0	0	0
Cinnamon	18	15 <sup>a</sup>	3	0	0
Garlic powder	16	16	0	0	0
Mustard powder	16	16	0	0	0
Oregano	15	9 <sup>a</sup>	2	2	2
TOTAL	114	101	9	2	2

<sup>a</sup>Two samples had 50/g

*C. perfringens* counts of 100/g and 250/g were found in two samples of cayenne pepper (Tables 1 and 3) from brands 2 and 3 (Table 5). Coliforms ranging from 40/g to 2400/g were found in four (27%) samples (Tables 4 and 6) from brands, 2, 3, and 9 (Table 5). No fecal coliforms or coagulase positive staphylococci were found at the lowest dilution tested and are reported as <30/g (Table 1).

TABLE 4. Spices containing food poisoning bacteria and coliforms

Spices	No. of samples	Percentage of spices containing		
		<i>C. perfringens</i>	Coagulase positive staphylococci	Coliforms
Bay leaves	16	12	0	0
Cayenne pepper	15	20	0	27 <sup>a</sup>
Chili powder	18	0	0	0
Cinnamon	18	28	0	22 <sup>a</sup>
Garlic powder	16	0	0	19 <sup>a</sup>
Mustard powder	16	0	0	0
Oregano	15	53	7	0

<sup>a</sup>Fecal coliforms were negative (< 30/g)

#### Chili powder

Eighteen samples of chili powder were analyzed. The APC ranged from 100/g to 540,000/g (Table 1), but only three samples had APC's greater than 100,000/g (Table 7). The yeast and mold count was less than 100/g in all samples except one which was 8100/g (Tables 1 and 2). None of the samples contained *C. perfringens*, coliforms, or coagulase positive staphylococci at the lowest dilution tested and are reported as <100/g or <30/g respectively (Table 1).

#### Cinnamon

Eighteen samples of ground cinnamon were analyzed. The APC ranged from 10,700/g to 780,000/g (Table 1), but only six samples had APC's greater than 100,000/g (Table 7). The yeast and mold count ranged from <100/g to 4600/g (Table 1) and half of the samples had counts between 100/g and 1000/g (Table 2). *C. perfringens* counts from 50/g to 300/g (Table 1) were found in five (28%) samples of cinnamon (Tables 3 and 4) from brands 1, 2 and 3 (Table 5). Coliforms ranging from 40/g to 230/g (Table 1) were found in four (22%) samples (Tables 4 and 6) of brands 2 and 3 (Table 5). No fecal coliforms or coagulase positive staphylococci were found at the lowest dilution tested and are reported as <30/g (Table 1 and 4).

#### Garlic powder

Sixteen samples of garlic powder were analyzed. The APC ranged from 3700/g to 980,000/g (Table 1) and half of the samples had APC's greater than 100,000/g (Table 7). Yeast and mold counts were less than 500/g (Table 1) and most were less than 100/g (Table 2). Coliforms were

found in two (19%) of the samples (Tables 4 and 6) of brand 3 (Table 5) with counts of 40/g and 230/g (Table 1). No *C. perfringens*, fecal coliforms, or coagulase positive staphylococci were found at the lowest dilution tested and are reported as <100/g and <30/g respectively (Table 1 and 4).

#### Mustard powder

Sixteen samples of mustard powder were analyzed. The APC was the lowest of all the spices, ranging from 500/g to 5300/g (Table 1). Yeast and mold counts were <100/g for 12 samples (Table 2) and ranged to only 2200/g (Table 1). No pathogens or coliforms were found in any of the samples of mustard powder at the lowest dilution tested and are reported as <100/g or <30/g as appropriate (Table 1).

#### Oregano

Fifteen samples of ground oregano were analyzed. The APC ranged from 1200/g to 3,600,000/g (Table 1). More than half of the samples had APC's less than 100,000/g (Table 7). The yeast and mold counts ranged from <100/g to 27,000/g (Table 1) and more than half of the samples had less than 10,000/g (Table 2). *C. perfringens* ranging from 50/g to 2850/g (Table 1) were found in 8 (53%) of the samples (Tables 3 and 4) from 4 different brands (Table 5). Thirty-three percent of the oregano samples containing *C. perfringens* were from brand 3 (Table 5). Coagulase positive staphylococci (30/g) were found in 1 (7%) sample of oregano (Tables 1 and 4) from brand 8 (Table 5). No coliforms were found in any of the oregano samples at the lowest dilution tested and are reported as <30/g (Table 1 and 4).

## DISCUSSION

Spices procured from the local supermarket by the Military and other consumers may contain as high as 9,100,000 bacteria (APC) per gram (Table 1). The APC varied widely between most samples of the same spice and between different spices (Table 7). However, with the exception of cayenne pepper, half or more of all the spice samples had APC's less than 100,000/g (Table 7). The APC may have no overt public health significance, but it may contribute to some degree toward spoilage of a

TABLE 5. Food poisoning bacteria and coliforms found in different brands of spice

Brand	Percentage of spices containing										
	Bay leaves		Cayenne pepper		Cinnamon		Garlic powder		Oregano		
	<i>C. perf</i> <sup>a</sup>	Coli. <sup>b</sup>	<i>C. perf.</i>	Coli.	<i>C. P. Staph.</i>						
1	0	0	0	0	11	0	0	0	0	0	0
2	0	0	7	7	5.5	11	0	0	7	0	0
3	6	0	13	13	11	5.5	0	19	33	0	0
4	0	0	—	—	—	—	—	—	—	—	—
5	0	0	0	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0	—	—	—
7	—	—	—	—	—	—	—	—	7	0	0
8	—	—	—	—	—	—	—	—	7	0	7
9	0	0	0	7	0	0	0	0	—	—	—
10	—	—	—	—	0	0	—	—	0	0	0

<sup>a</sup>*C. perfringens*

<sup>b</sup>Coliforms

TABLE 6. *Coliform count of processed spices used by the Armed Forces*

Spices	No. of samples	Number of samples containing (MPN per gram)			
		<30	30 to 100	101 to 230	>230
Bay leaves	16	16	0	0	0
Cayenne pepper	15	11	3	0	1 <sup>a</sup>
Chili powder	18	18	0	0	0
Cinnamon	18	14	3	1	0
Garlic powder	16	14	1	1	0
Mustard powder	16	16	0	0	0
Oregano	15	15	0	0	0
TOTAL	114	104	7	2	1

<sup>a</sup>2400/g

product in which a spice is used (7). The APC may also indicate to what extent the finished spice has been cleaned and whether or not it has been mishandled. There was no correlation between APC and yeast and mold counts.

Only five samples of spice, representing three different kinds, had yeast and mold counts greater than 10,000/g (Table 2). These counts were considerably lower than found in black and red pepper (3). An exception was one sample of bay leaves which had a yeast and mold count of 670,000/g (Tables 1 and 2). Whether such a spice, which appears to be an exception, poses a health hazard is a question since aflatoxin has not been demonstrated in spices. However, it cannot be considered wholesome and large numbers of toxigenic molds have been found in spices (3, 4).

Low numbers of coliforms were found in cayenne pepper, cinnamon, and garlic (Table 1 and 6), representing 9% of the samples analyzed. The counts equalled or exceeded 100/g in only three samples (one of each kind). Their presence does not indicate a health hazard since fecal coliforms were not found at the lowest dilution tested. However, coliforms are not a necessary ingredient of spices since they were found in only three kinds of spice (Table 4 and 6) and in only two brands out of 10 (Table 5). The occurrence of *E. coli* in spices is apparently rare and very sporadic (7). Preliminary studies (see Materials and Methods) showed that growth of *E. coli* was not inhibited by the concentration of spices tested, or by the procedures employed.

Since salmonellae and shigella are rarely, if ever, found in spices (3, 7) the only pathogens searched for in this study were *Staphylococcus aureus* and *C.*

*perfringens*. Coagulase positive staphylococci were found in only one sample of spice (oregano) and at a very low level (Table 1 and 4). Their low incidence and low numbers in the spices analyzed indicate that they do not pose a health hazard and are not commonly found in spices. Preliminary studies (see Materials and Methods) showed that growth of *S. aureus* was not inhibited by the spice concentration tested or by the procedures employed. The modified MPN procedure employed favored the recovery of injured staphylococci, so it is not likely that viable staphylococci were present, but were unable to grow.

The incidence of *C. perfringens* in the spices analyzed was 15% (17 samples) as opposed to 6% or less in other American foods and pepper (11). The counts ranged from 50/g to 2850/g (Table 1). *C. perfringens* was found in four out of seven types of spices (Table 4), but in only two brands (Table 5). *C. perfringens* was found in more than half of the oregano samples analyzed, but in 33% of oregano from brand 3 (Table 5). Since *C. perfringens* is a food poisoning organism, its presence in spices is potentially hazardous to health. The spores may survive cooking temperatures and will grow in foods at temperatures between 20-50 C (68-122 F). It is also an indicator of fecal pollution in some European countries (8).

Except for the relatively high incidence of *C. perfringens* found in some spices, oregano in particular, this study would support the conclusion of a recent investigation that there was no potential health hazard associated with spices and herbs (6). However, the investigators did not search for *C. perfringens*.

Spices harboring *C. perfringens* must be considered a potential health hazard because they (and other pathogens) may grow in foods which are seasoned or garnished with spice if the food is not adequately cooked or properly refrigerated.

The support by others of the reported demand by the Consumer Union (4) for rigorous standards for cleanliness of spices is probably justified. Although many industries police their own products, apparently some do not (Table 5).

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TABLE 7. *Aerobic plate count (APC) of processed spices used by the military*

Spices	No. of samples	Number of samples containing (per gram)								
		<100	100 to 1000	1001 to 10,000	10,001 to 50,000	50,001 to 100,000	100,001 to 500,000	500,001 to 1,000,000	1,000,001 to 5,000,000	5,000,001 to 10,000,000
Bay leaves	16	1	0	7	8	0	0	0	0	0
Cayenne pepper	15	0	0	1	1	0	3	1	4	5
Chili powder	18	0	6	3	4	2	2	1	0	0
Cinnamon	18	0	0	0	9	3	4	2	0	0
Garlic powder	16	0	0	4	2	2	5	3	0	0
Mustard powder	16	1	6	9	0	0	0	0	0	0
Oregano	15	0	0	5	4	2	1	2	1	0
TOTAL	114	2	12	29	28	9	15	9	5	5

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