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EFFECTS OF MICROWAVES ON BACTERIA IN FROZEN FOODS

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FOREWORD

This study was performed by personnel of the Microbiology-Immunology Branch, Biosciences Division, under task No. 775309 in conjunction with the Base Veterinary Laboratory, Francis E. Warren Air Force Base, Wyo. The work was accomplished between 15 September 1969 and 23 May 1970. The paper was submitted for publication on 2 September 1970.

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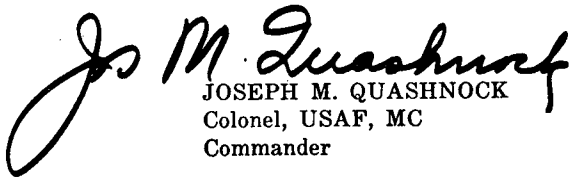

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ABSTRACT

The effects of freezing and microwave heating on deliberately contaminated precooked frozen meal components were studied. Freezing conditions and food type appeared to influence survival of *Escherichia coli* as time increased, but *Streptococcus faecalis* exhibited no apparent change. Twenty-seven groups of samples representing 17 different foods were analyzed. The results suggest that the microwave heating will satisfactorily kill or reduce incidental and introduced microorganisms to a safe level provided the microwave exposure time is correlated with the size and type of food substance being treated.

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I. INTRODUCTION

The frozen foil pack meal system was developed to support Strategic Air Command missile-site crews. A previous study (1) has shown that it is feasible to produce frozen food components of foil pack meals which are microbiologically acceptable under the provisions outlined in the military specifications governing precooked frozen foods (2). However, accidental contamination with pathogenic bacteria can occur when the precooked foods are packaged. Microwave ovens are being considered for heating these foods, since these units are known to have a bactericidal effect (3, 4, 5). The objectives of this effort were to determine the effects of freezing on inoculated marker organisms and to study the effects of microwave treatment on total numbers of aerobic bacteria, as well as on the marker organisms.

II. MATERIALS AND METHODS

Precooked food items obtained from the Foil Pack Kitchen were deliberately contaminated with "marker" bacteria and then frozen by Base Veterinary Office personnel at Francis E. Warren AFB, Wyo. Fifteen samples of each food item to be tested were shipped on Dry Ice to the USAF School of Aerospace Medicine for analysis each week. Six of the samples were inoculated with *Escherichia coli;* 6 were inoculated with *Streptococcus faecalis;* and the remaining 3 samples were left uninoculated to serve as controls. In this way, the normal level of contamination was compared with that of introduced marker organisms. Half of the inoculated samples (3 with *E. coli* and 3 with *S. faecalis)* served as controls in determining the efficiency of the recovery medium, and in measuring the effects of freezing at −20° C. for 3 to 6 days before making analyses. The remaining 6 samples (3 with *E. coli* and 3 with *S. faecalis)* were exposed to microwave energy (2,450 mc.) in a Litton model 550 microwave oven. Three different treatment cycles were used: 15 seconds for thawing, 60 seconds resting, and 15 seconds for heating; 30 seconds for thawing, 90 seconds resting, and 30 seconds for heating; and 45 seconds for thawing, 120 seconds resting, and 45 seconds for heating. The rest period allowed ice crystals within the food to melt between thawing and heating.

Bacteriologic analyses were performed as recommended by the Association of Food and Drug Officials of the United States (6). The entire sample was homogenized in a Waring Blendor with sterile buffered (pH 7) distilled water to make a slurry. The slurry was serially diluted to 10^{-5} with the buffered water. Total counts of aerobic bacteria, using plate count agar (Difco), were made on all samples. The direct plate method, using violet-red bile agar (VRB) (Difco), and the most-probable-number (MPN) method, employing lauryl sulfate tryptose broth (Difco), were used for the recovery of *E. coli. S. faecalis* organisms were recovered by the direct plate method, using KF agar (Difco), and by the MPN method, using KF broth (Difco).

III. RESULTS AND DISCUSSION

Because of unforeseen shut-downs at the Foil Pack Kitchen facility at F. E. Warren AFB, it was not possible to obtain the number of samples and the variety of foods desired for study.

TABLE I

Effects of freezing on E. coli *in contaminated foods*

Sample No.	Food item	Days frozen	Average wt. in grams (3 samples)	Number of marker organisms per gram (3 samples)	Number recovered per gram		Percent reduction	
					Direct plate method	MPN* method	Direct plate method	MPN* method
1	Macaroni and cheese	5	51.8	211	3	<4	98.6	98.3
2	Macaroni and cheese	5	52.5	46,000	2,900	6,700	93.7	84.4
3	Macaroni and cheese	6	138.0	20,000	53	930	99.7	95.4
4	Macaroni and cheese	6	98.8	180,000	2,000	11,000	99.9	93.9
5	Macaroni and cheese	6	85.5	160,000	820	4,200	94.9	97.4
6	Macaroni and cheese	6	113.3	120,000	300	14,000	99.8	88.3
7	Baked ham	5	105.7	19,000	1,200	>1,100	93.7	†
8	Baked ham	6	86.7	30,000	3,700	6,700	87.7	77.7
9	Baked ham	4	48.3	650,000	24,000	>110,000	96.3	†
10	Spaghetti	5	60.8	147	90	234	38.8	†
11	Spaghetti	5	98.5	16,000	5,400	8,800	66.3	45.0
12	Buttered corn	5	51.2	2,700	316	887	88.3	67.2
13	Buttered corn	5	52.2	17,000	5,600	>1,100	67.1	†
14	Roast beef	6	66.0	270,000	49,000	110,000	81.9	59.3
15	Roast beef	5	109.8	120,000	20,000	9,000	83.3	92.5
16	Peas and carrots	5	59.8	157	77	93	50.9	40.8
17	Mashed potatoes	5	60.0	162	114	350	29.6	†
18	Mixed vegetables	5	55.2	2,200	64	93	97.1	95.8
19	Succotash	5	114.2	18,000	240	4,900	86.7	72.8
20	Turkey pot pie	5	109.7	13,000	123	700	99.1	94.6
21	Fried fish sticks	5	45.8	33,000	5,100	6,700	84.6	79.7
22	Meat balls	6	116.8	160,000	17,000	60,000	89.4	62.5
23	Beef pot pie	4	104.2	310,000	0	0	†	†
24	Chicken pot pie	3	131.3	160,000	†	88,000	†	†
25	Fried chicken	3	22.3	920,000	390,000	>110,000	57.6	†
26	Chili	3	94.7	130,000	88,000	88,000	32.3	32.3
27	Boston baked beans	3	92.2	130,000	130,000	>110,000	0.0	†

*MPN = most probable number.

†No data obtained.

The effect of cold on *E. coli* was quite dramatic (table I). Reduction ranged from a high of 99.9% in macaroni and cheese (sample 4) which had been frozen 6 days, to a low of 0.0% in Boston baked beans (sample 27) which had been frozen for 3 days. Although insufficient samples were analyzed to be statistically significant, the data suggest that increased freezing time and food consistency may have influenced the number of bacteria surviving during storage. Because of a low recovery rate for *E. coli* in the early stages of the study, it was necessary to increase the level of contamination from an initial 10^2 organisms per gram of sample to an inoculum of 10^5 organisms per gram. The larger inoculum was used during the major portion of the study to assure survival of enough marker organisms to adequately evaluate the effect of microwave energy.

S. faecalis counts remained the same or were higher after freezing. This might be attributed, in part, to fragmentation of the streptococcus chains during the homogenizing process of the tests, but this organism also seems to resist the cold temperatures encountered in freezing. No tabular data are included on *S. faecalis* since there was no apparent deleterious effect of freezing on this organism.

During the early stages of the study each treatment cycle consisted of a 15-second microwave exposure for thawing, a 60-second rest period, and another 15-second microwave exposure for heating. Although the food samples were relatively small (less than 60 gm.), this treatment was not completely effective in killing the marker and indigenous organisms. When thawing and heating exposures were increased to 30 seconds each with a 90-second rest period, the treatment still was not adequate to kill the increased concentration of marker organisms inoculated into the foods. In order to obtain more useful data, the size of food samples was increased to a full serving (up to 145 gm. in some individual portions) and the treatment was increased to 45 seconds each for thawing and heating, with a 120-second rest period.

Results of the microwave treatment on total numbers of bacteria in contaminated foods are shown in table II. In tabulating these data, duplicate plates averaging less than 30 colonies per plate in the lowest dilution were recorded as having less than 300 organisms per gram. Except for samples 7 and 9, the uncontaminated controls had relatively low total plate counts. Data on treated samples 10 and 18 were unsatisfactory owing to problems encountered in the test system. Of the 25 samples yielding usable data, 11 had greater than 300 organisms per gram after microwave treatment. Of these, 5 (samples 2, 12, 13, 16, and 17) received one of the two shorter microwave treatments, and the remaining 6 were relatively large samples in which a longer exposure would probably have achieved complete killing. The other 14 samples included 11 items in relatively small portions, and 3 items (samples 15, 19, and 23) in larger portions. These latter samples had high water content and were therefore more conducive to efficient heating. Organisms surviving the longer microwave exposures were mainly spore-formers, which are not as susceptible to the heat levels attained as are vegetative cells (4).

As shown in table III, microwave treatment was generally effective in killing the marker bacteria. Five samples yielded *E. coli* after treatment, but one (sample 22) gave such contradictory results between the direct plate method and the MPN method that the results from the sample must be discounted. It was anticipated that the MPN method would routinely give higher counts than the direct plate method because of the apparently higher degree of sensitivity of the MPN method (7, 8, 9). Inconsistent results were also noted in the recovery of *S. faecalis* from samples 1, 2, and 6. The remaining 3 samples yielded results more in line with quantitative relationships expected when marker organisms were recovered after treatment. It should be noted that treated samples yielding marker organisms were among those giving elevated total plate counts, or were larger portions, or were those exposed to short microwave treatment.

TABLE II

Effects of microwaves on total numbers of bacteria in contaminated foods

Sample No.	Food item	Average weight (15 samples)	Type of microwave treatment*	Total bacteria per gram in samples				
				Uncontaminated (Controls)	Contaminated with *E. coli*		Contaminated with *S. faecalis*	
					Untreated	Treated	Untreated	Treated
1	Macaroni and cheese	56.6	1	<300	<300	<300	1,500	<300
2	Macaroni and cheese	50.5	2	<300	12,000	400	5,600	400
3	Macaroni and cheese	126.4	3	450	16,000	350	24,000	400
4	Macaroni and cheese	99.4	3	<300	200,000	<300	48,000	<300
5	Macaroni and cheese	97.1	3	<300	2,600	<300	47,000	<300
6	Macaroni and cheese	113.6	3	<300	180,000	14,000	42,000	<300
7	Baked ham	110.9	3	330,000	480,000	1,200	46,000	5,300
8	Baked ham	84.9	3	<300	28,000	<300	46,000	<300
9	Baked ham	53.4	3	130,000	550,000	<300	500,000	<300
10	Spaghetti	60.0	2	1,400	2,200	†	2,500	†
11	Spaghetti	85.3	3	<300	19,000	<300	33,000	<300
12	Buttered corn	51.9	2	4,800	5,500	2,100	11,000	630
13	Buttered corn	50.9	2	9,800	18,000	2,100	11,000	640
14	Roast beef	61.3	3	840	320,000	<300	36,000	<300
15	Roast beef	114.0	3	5,000	16,000	<300	48,000	<300
16	Peas and carrots	54.8	1	3,600	4,100	550	3,100	550
17	Mashed potatoes	54.7	1	1,200	1,000	450	1,200	510
18	Mixed vegetables	51.1	2	†	†	†	†	†
19	Succotash	111.8	3	<300	20,000	<300	59,000	<300
20	Turkey pot pie	110.6	3	4,700	10,000	670	33,000	840
21	Fried fish sticks	46.1	3	10,000	38,000	<300	55,000	<300
22	Meat balls	117.3	3	10,000	190,000	20,000	67,000	1,100
23	Beef pot pie	98.2	3	<300	300	<300	67,000	<300
24	Chicken pot pie	132.4	3	910	730,000	12,000	60,000	720
25	Fried chicken	24.9	3	4,000	>2,000,000	<300	510,000	<300
26	Chili	99.0	3	<300	550,000	<300	72,000	<300
27	Boston baked beans	94.3	3	5,500	800,000	<300	73,000	<300

*Microwave treatment: 1 = 15 sec. thaw, 60 sec. rest, 15 sec. heat; 2 = 30 sec. thaw, 90 sec. rest, 30 sec. heat; 3 = 45 sec. thaw, 120 sec. rest, 45 sec. heat.

†No data obtained.

TABLE III

Effects of microwaves on E. coli *and* S. faecalis *in contaminated foods*

Sample No.	Food item	Average weight (12 samples)	Type of microwave treatment*	Contaminated controls				Microwave-treated			
				E. coli per gram		*S. faecalis* per gram		*E. coli* per gram		*S. faecalis* per gram	
				Direct plate method	MPN† method	Direct plate method	MPN† method	Direct plate method	MPN† method	Direct plate method	MPN† method
1	Macaroni and cheese	55.2	1	3	<4	672	600	0	0	0	45
2	Macaroni and cheese	49.0	2	2,900	6,700	4,500	3,900	820	2,300	5,000	980
3	Macaroni and cheese	126.7	3	53	930	28,000	31,000	0	0	0	0
4	Macaroni and cheese	100.5	3	2,000	11,000	44,000	66,000	0	0	0	0
5	Macaroni and cheese	97.4	3	820	4,200	50,000	31,000	0	0	0	0
6	Macaroni and cheese	113.0	3	300	14,000	40,000	67,000	73	2,300	8	0
7	Baked ham	109.3	3	1,200	>1,100	74,000	53,000	0	0	0	0
8	Baked ham	85.6	3	3,700	6,700	33,000	26,000	0	0	0	0
9	Baked ham	55.1	3	240,000	>110,000	220,000	>110,000	0	0	0	0
10	Spaghetti	60.5	2	90	234	541	1,100	0	0	0	0
11	Spaghetti	87.6	3	5,400	8,800	27,000	>11,000	0	0	0	0
12	Buttered corn	51.1	2	316	887	455	527	0	0	0	0
13	Buttered corn	51.0	2	5,600	>1,100	3,400	>1,100	0	0	0	0
14	Roast beef	62.6	3	49,000	110,000	31,000	31,000	0	0	0	0
15	Roast beef	113.8	3	20,000	9,000	41,000	67,000	0	0	0	0
16	Peas and carrots	55.3	1	77	93	283	600	0	0	0	0
17	Mashed potatoes	55.6	1	114	350	418	600	0	0	2	12
18	Mixed vegetables	47.6	2	64	93	531	460	0	0	0	0
19	Succotash	112.5	3	240	4,900	54,000	60,000	0	0	0	0
20	Turkey pot pie	111.3	3	123	700	22,000	>11,000	0	30	201	238
21	Fried fish sticks	46.0	3	5,100	6,700	40,000	39,000	0	0	0	0
22	Meat balls	117.2	3	17,000	60,000	26,000	16,000	0	38,000	0	0
23	Beef pot pie	114.0	3	0	0	70,000	67,000	0	0	0	0
24	Chicken pot pie	131.1	3	1	88,000	45,000	67,000	22	180	300	460
25	Fried chicken	25.7	3	390,000	>110,000	350,000	>110,000	0	0	0	0
26	Chili	99.4	3	88,000	880,000	81,000	46,000	0	0	0	0
27	Boston baked beans	83.5	3	130,000	>110,000	72,000	67,000	0	0	0	0

*Microwave treatment: 1 = 15 sec. thaw, 60 sec. rest, 15 sec. heat; 2 = 30 sec. thaw, 90 sec. rest, 30 sec. heat; 3 = 45 sec. thaw, 120 sec. rest, 45 sec. heat.

†MPN = most probable number.

IV. CONCLUSIONS

Although an insufficient number of samples were analyzed to be statistically significant, the results suggest that the microwave oven will satisfactorily kill or reduce incidental and introduced microorganisms in these meal components to a safe level provided the microwave exposure time is properly correlated with the size and type of food substance being treated.

Additional tests based on the size and type of food are needed to determine the exposure times required to assure microbiologic safety of these meal components.

Damage to *E. coli* resulting from storage at freezer temperatures appears to be related to time and food types, but recovery rates for *S. faecalis* indicate that that organism is resistant to freezing conditions.

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13. ABSTRACT

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