Heat Resistance of Salmonella in Dried Milk

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Abstract

The effects of heat, moisture, storage temperatures, and chemical additives on the growth and survival of salmonellae in nonfat dried milks were determined. Artificially contaminated powders of various moisture levels were subjected to tests which included storage at temperatures of 4.4 to 50 C, storage with chemical additives, exposure of thin layers to oven heat ranging from 60 to 115 C, boiling of fixed beds of powder with hot air streams at 87.7 to 148.8 C, and determination of time-temperature necessary for destruction of salmonellae in fluid and concentrated milks. Moisture levels and storage temperatures influenced growth and survival, and salmonellae added to nonfat dried milk were quite resistant to dry heat.

Although salmonellosis has long been recognized as a public health problem, interest has grown during the past 10 years as increased numbers of outbreaks have been identified. A recent estimate claims that salmonellosis now affects more people—an estimated million in the United States, than any other disease (1). While the foods most commonly contaminated are meat and poultry products, dairy products have also been implicated. In 1965 an outbreak was traced to instant nonfat dried milk (NDM) (5). Subsequently the U.S. Department of Agriculture, U.S. Public Health Service, and U.S. Food and Drug Administration made independent surveys for salmonellae in dried milks. The Food and Drug Administration reported 3 to 5% of powders tested as positive, USDA found 2% of 2000 samples positive, and USPHS found no positives in 250 samples (3).

Pasteurization kills salmonellae in fluid milk; thus its presence in dried milk indicates post-pasteurization contamination. Information is lacking concerning survival and growth characteristics in milk powders during manufacture and storage under both normal and adverse conditions. This paper presents results of experiments designed to show the effects of heat, moisture, storage, and chemical additives on the growth and survival of salmonellae in NDM.

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Experimental Procedures [1]

Cultures. Three test species of *Salmonella* were selected on the basis of their reported heat resistance and frequency of occurrence in dairy products. They were *Salmonella senftenberg* 775W, *Salmonella typhimurium* TM1, and *Salmonella new brunswick* 1608. The cultures were propagated in trypticase soy broth at 37 C for 18 hr before each use.

Preparation of powders. Powders used for all experiments were low-heat NDM obtained from a local supply house. A control batch was tested for bacteriological quality and was then artificially contaminated with a mixture of the organisms named above and stored at 4.4 C until ready for use. Batches of powders were contaminated by two methods: (a) Large quantities were prepared by using a compressed air atomizer to spray a fine mist of culture into the powder through a small opening in a laboratory size, elbow shaped, Patterson-Kelly rotating blender. (b) Smaller quantities were prepared by grinding packed cells from centrifuged cultures into the powder with a mortar and pestle. Sufficient amounts of culture were added by each method to give powders containing approximately 10^3 to 10^4 organisms/g. All powders were prepared under an enclosed hood using sanitary precautions to prevent contamination of the atmosphere.

Storage tests. The effect of storage temperature on the survival of salmonellae in NDM was determined by storing contaminated powders prepared as previously described in sealed jars at temperatures ranging from 4.4 to 50 C. In addition, four samples were frozen at −17 C and thawed at 22.5 C several times during storage to test the effect of thermal shock.

Selected chemical additives, i.e., sodium benzoate, dimethyl pyrocarbonate, and potassium sorbate were studied for their effects on survival of salmonellae in NDM during storage. Test chemicals were added to contaminated

[1] Reference to certain products or companies does not imply an endorsement by the Department over others not mentioned.

skimmilk concentrates which were freeze dried, placed in glass jars, and stored at 22.5 C. Samples from each experiment were tested at frequent intervals for survival of salmonellae.

Moisture tests. The effects of moisture on both growth and survival of salmonellae were determined in powders ranging from 4 to 15% moisture and in concentrates ranging from 20 to 50% solids. Powders were adjusted to the desired moisture levels by mist spraying a known volume of water into the powder while mixing and allowing the powders to reach equilibrium. Concentrates were prepared by weighing powder and water into plastic bags and kneading until a homogeneous mixture was reached. Powders wetted to 4, 7, 15, 20, 30, 40, and 50% were incubated at 37 C for three days. The progress of salmonellae growth was determined daily.

Heat inactivation tests on powders. The amount of heat required to destroy salmonellae in normal and high moisture powders was determined by two methods, a) exposure of thin layers of contaminated powder to thermostatically controlled dry heat, and b) passing controlled hot air through a fluidized bed of NDM under conditions comparable to a commercial after-dryer.

Samples of contaminated powder were placed in 30.5-cm square aluminum pans to a depth of 0.6 cm, and were heated for various time intervals in a dry heat oven at temperatures of 60, 76.6, 85, and 115.5 C. High moisture samples ranging from 7 to 25% were heated at 85 and 115.5 C. Samples were removed at frequent intervals and tested for salmonellae.

The commercial drying phase of the instantizing process was simulated in a laboratory after-dryer. It consisted of a drying chamber made up of a 10.2 cm OD sintered glass funnel with an attached side arm and a 10.2 cm OD by 122-cm long plexiglass tube connected to the funnel by a rubber sleeve. The tube was used as a chimney to extend the drying chamber and reduce the loss of powder particles. The side arm served as an air inlet. A chromel alumel thermocouple was inserted into the stem of the funnel through a rubber septum to record inlet air temperature. Outlet air temperature was determined with a thermistor inserted through the wall of the chimney, 30.5 cm above the sintered glass disc. Pressurized compressed air or CO_2 was heated to the desired temperature by passing it through 305 cm of 0.6-cm copper tubing coiled and immersed in a hot oil bath. Air temperatures of 87.7 to 148.8 C for 3-6 min were used to dry 100 g lots of powder agglomerated to 7, 10, 15, and 25% moisture by the previously described method.

Heat inactivation tests on concentrate. Comparative studies were made to determine time-temperature combinations necessary to give negative results for salmonellae in normal milk (10% solids) and concentrated milk (50% solids), each inoculated with 10^5 to 10^7 organisms per gram. Beakers containing 250 ml portions of each were placed in a large glass water bath on a magnetic stirrer and were constantly mixed with a stirring bar. The milks were brought to temperature before addition of the test organisms. *S. senftenberg* (775W) and *S. new brunswick* (1608) were tested at temperatures of 54.4, 61.6, and 65.5 C for times ranging from 1 to 50 min.

Salmonella determinations. Salmonellae determinations were made by direct plating on a modified Lysine-Iron Agar (2). All negative results obtained by direct plating were confirmed by adding 10 g of test powder or concentrate to enrichment broths, incubating at 37 C for 24 hr and streaking on Brilliant green agar.

Results and Discussion

Effects of storage. Survival of salmonellae during storage at temperatures of 4.4 to 50 C is shown in Table 1. The storage temperature of NDM had a marked effect on survival. Little drop in viable count was observed in powders

TABLE 1. Survival of salmonella in contaminated NDM during storage.

Storage time	Temperature (C)					
	4.4	15.5	26.6	37.7	43.3	50.
(weeks)	(Salmonella/g)					
0	220×10^4	220×10^4	220×10^4	220×10^4	16×10^4	16×10^4
1	170×10^4	85×10^4	110×10^4	49×10^3	9×10^3	4×10^2
2	155×10^4	120×10^4	55×10^4	37×10^3	27×10^1	30×10^1
3	9×10^4	36×10^4	79×10^3	29×10^3	11×10^1	1×10^1
4	65×10^4	50×10^4	30×10^3	20×10^3	10×10^1	8
6	69×10^4	25×10^4	35×10^3	12×10^3	10	1
15	20×10^4	40×10^3	30×10^2	16×10^2	....	....

TABLE 2. Effect of moisture on growth and survival of salmonella in NDM at 37 C.

Moisture	(Salmonella/g) 0 hr	24 hr	72 hr
(%)			
4	92×10^3	23×10^3	11×10^3
7	92×10^3	86×10^2	70×10^2
15	92×10^3	9×10^1	1×10^1
20	92×10^3	18×10^1	4×10^1
30	92×10^3	10×10^2	7×10^2
40	92×10^3	21×10^3	21×10^5
50	92×10^3	80×10^6	120×10^7

stored for 15 weeks at the lower temperatures; however, the rate of destruction increased with increasing storage temperature. Alternate freezing and thawing of powders did not appear to hasten the rate of destruction. It is doubtful that a high storage temperature can be used successfully to eliminate salmonellae contamination without adversely affecting the flavor of the product.

Effect of food preservatives. None of the food preservatives known to inhibit salmonellae in the liquid state had any significant effect on their survival in the dry state. Drops in viable count were the same in the control and in powders to which 0.1% diethylpyrocarbonate, 500 ppm potassium sorbate, 0.2% sodium benzoate, and 1 and 10% whey had been added, and stored at 22.5 C for up to 3 months.

Effect of moisture. The influence of moisture on growth and survival of salmonellae at 37 C is shown in Table 2. The drop in viable count was correlated closely with a rise in moisture up to about 15 to 20%. Beyond 20%, a leveling off of destruction occurs with good growth beginning at 40%. These results and the storage results (Table 1) indicate that unfavorable levels of moisture may interrupt the dormant conditions necessary for survival.

Heat resistance of Salmonellae in powder. The extreme resistance of salmonellae to destruction by dry heat is demonstrated in Table 3. Neither 60 or 76.6 C gave sufficient killing after 10 hr. This may be compared with the results of Rasmussen who showed that 76.6 C killed salmonellae in dried feather meal in 15 min (4). However, the moisture content of the meal was 8.2% compared to 4% for NDM. He found heat resistance of salmonellae to vary greatly in different meals thereby suggesting that moisture and other factors play an important role. The degree of heat required for destruction in a short time (115.5 C–1 hr) was too intense and imparted a yellow burnt appearance to the powder.

The importance of moisture in destruction by heat is evident from results in Figure 1. Two hours were insufficient to give negative results in 4 and 7% powders at 85 C, though 30 min was enough at the 25% level. The time of heating at 115.5 C required to give negative results was only about half the time required at 85 C.

Results of drying powders of different moisture levels in a laboratory after-dryer are shown in Table 4. Although commercial methods do not usually exceed 115.5 C or 14% moisture, temperatures of up to 148.8 C and moistures of up to 25% were tested. Here again, high moistures increased the rate and extent of killing. The increase in lethal effects reached a maximum around the 15% moisture level. It is probable that since moisture higher than 15% formed larger agglomerates, the rate of heat conductance to the center of the larger agglomerates was slowed. Salmonellae was not detected in powders containing 15% moisture, treated with a time-temperature combination of 148.8 C for 6 min; however, the powder particles were charred and had a cooked odor and appearance. It was assumed that the fluidized bed method would be more effective than the thin layer method due to increased efficiency of moving air. However, a comparison of results

TABLE 3. Dry heat resistance of salmonella in nonfat dried milk.[a]

Exposure	60 C	76.6 C	85 C	115.5 C
		(Salmonella/ml[b])		
0	69×10^4	73×10^4	94×10^3	94×10^3
15 min	54×10^4	....	....	16×10^3
30 min	45×10^4	135×10^3	71×10^2	8×10^2
45 min	....	....	....	2×10^1
1 hr	47×10^4	45×10^3	87×10^1	<1
2 hr	30×10^4	50×10^3	35×10^1	<1
3 hr	38×10^4	30×10^2	8×10^1	<1
4 hr	....	29×10^2	5×10^1	<1
5 hr	30×10^4	14×10^2	2	<1
10 hr	40×10^2	35×10^1	0	<1

[a] Thin layers of conventional (4% moisture) powder heated in oven.
[b] Negative results from 10 g samples recorded as <1.

TABLE 4. Effect of forced air heating on survival of salmonella.[a]

Exposure			
Temperature	Time	Moisture	Salmonella[b]
(C)	(min)	(%)	(no./g)
......	0	4	116×10^3
......	0	7	112×10^3
......	0	10	109×10^3
......	0	15	104×10^3
......	0	25	95×10^3
87.7	3	4	31×10^3
87.7	3	7	23×10^3
87.7	3	10	20×10^3
87.7	3	15	10×10^2
87.7	3	25	20×10^2
98.8	3	4	56×10^3
98.8	3	7	22×10^3
98.8	3	10	27×10^2
98.8	3	15	20×10^2
98.8	3	25	30×10^2
115.5	3	4	150×10^2
115.5	3	7	180×10^2
115.5	3	10	160×10^2
115.5	6	10	100×10^2
115.5	3	15	24×10^2
115.5	6	15	50×10^1
115.5	3	25	30×10^2
115.5	6	25	15×10^2
148.8	3	4	30×10^3
148.8	6	4	20×10^3
148.8	3	15	20×10^1
148.8	6	15	<1

[a] Bubbled with hot air in laboratory after-dryer.
[b] Negative results from 10 g samples recorded as <1.

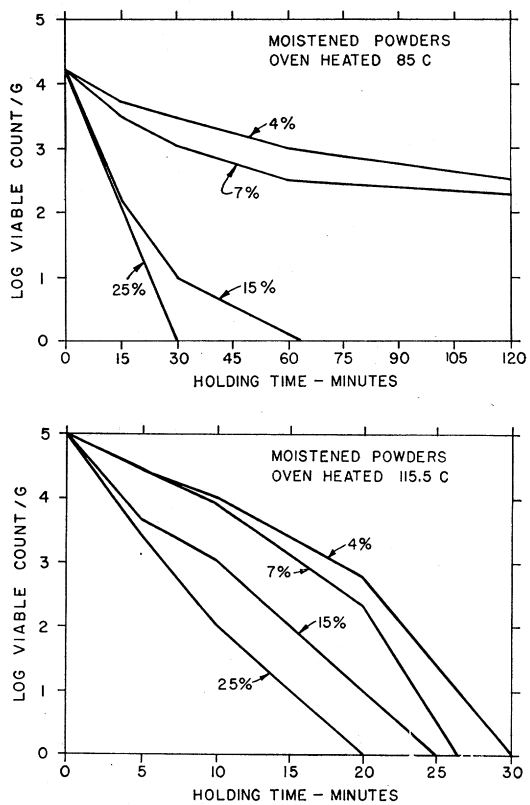


FIG. 1. Influence of moisture level on thermal destruction of Salmonella in nonfat dried milk at 85 and 115.5 C.

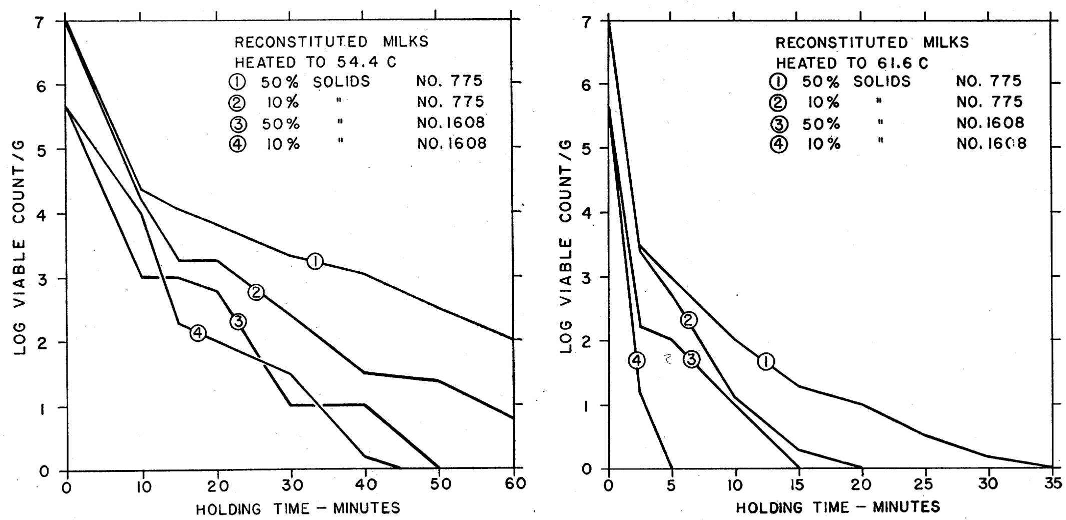
in Table 4 and Figure 1 do not show significant differences in lethal effects during comparable time intervals. Thus the results indicate that commercial after-dryers cannot be relied upon to "sanitize" contaminated powder.

Literature reports on food preservation have emphasized the germicidal effect of CO_2. No appreciable difference was noted when hot CO_2 gas was used in place of air in the fixed bed drying operation.

It appears that although certain combinations of heat and moisture will aid in lessening chances of salmonellae surviving during the drying operation, they cannot be relied upon for complete control. It may be assumed, however, that natural contamination would not approach the levels used here, and treatment necessary for destruction could be less severe than indicated in these experiments.

Heat resistance of Salmonellae in fluid and concentrated milk. Figure 2 compares results of time-temperature studies on skimmilk (10%) and concentrate (50%). The increase in heat tolerance of 775W is in agreement with general knowledge. In addition, survival of all species was greater in concentrated than in the fluid milk. *S. senftenberg* survived 65.5 C for 6 min in the concentrate but negative results were obtained for samples of skim treated 3 min at this temperature. The results correspond with accepted knowledge that salmonellae are easily killed by pasteurization in fluid milk; this study indicates that higher temperatures will be needed in concentrates. This fact is significant when considering requirements for concentrates which are intershipped among plants for further processing.

The importance of sanitation for control of salmonellae in food processing has been established. It is evident that acceptable alterations in present processing and storage methods offer little possibility for effective control. Thus, this study reemphasizes the need for proper sanitation during the concentrating, agglomerating, and drying of NDM. These results provide a reasonable indication of the extreme dry heat resistance of salmonellae and supply information that may be of value in determining potential trouble spots during manufacture.



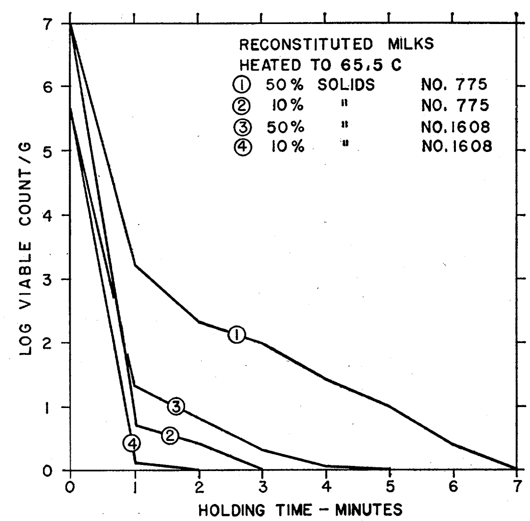


FIG. 2. Thermal destruction of *Salmonella senftenberg* 775W, and *Salmonella new brunswick* 1608 in fluid (10% solids) and concentrated (50% solids) milks.

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