

Dry Heat Destruction of Spores on Metal Surfaces and on Potatoes During Baking

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ABSTRACT

Heat destruction characteristics of the normal microflora on potatoes and of *Bacillus subtilis* var. *niger* spores deposited on potatoes were determined during heating in an air oven at 175 C. These results were compared to the heat destruction characteristics of *B. subtilis* var. *niger* deposited in metal cups heated at several temperatures in the same oven. The results of this study indicate that; (a) *B. subtilis* var. *niger* spores in tin cups have a D(150 C) of 0.92 min. and a z-value of 21.8 C, (b) *B. subtilis* var. *niger* spores on potato surfaces are more resistant to dry-heat destruction than when they are on metal surfaces, and (c) the normal microorganisms on potatoes are less heat resistant than *B. subtilis* spores on potato surfaces. Results of this study suggest that the normal flora of a potato are not eliminated during baking and that a spore population inoculated by chance onto a potato also will likely survive the baking process.

Recent environmental microbiological studies indicate that microbial spores under optimum humidity or moisture conditions are very difficult to inactivate. What is the significance of these findings in the food processing or food preparation area? What are the destruction rates of spores on a food product that is prepared by heating in a dry heat environment? Are the spores on the skin of a potato that is prepared by baking in a hot air oven destroyed by the heat treatment? If large numbers of pathogenic microorganisms were accidentally deposited on potatoes that were subsequently baked and consumed, would these organisms constitute a hazard? This study was carried out to obtain data that would make it possible to reach some conclusions regarding the sporicidal effect of a dry heat treatment applied to the surface of a potato or organic material containing considerable amounts of water that will diffuse to the surface and evaporate during the heating process. Since many individuals avidly consume the potato skin along with the flesh of the potato, it seemed desirable to determine the microbial destruction on the surface of the potato during the baking process.

Sterilization is the complete removal or killing of all microbial life. It is common practice to divide heat

sterilization into "wet-heat" and "dry-heat." In wet-heat sterilization processes, water in the liquid state is present in the system. In dry-heat sterilization processes, the quantity of water is not zero, but the substrate is not wet. Unless heroic measures are employed there will always be some moisture present regardless of the system or item. Therefore, dry-heat sterilization is a heat process in which the quantity of water in the system ranges from a relative humidity value near 0% to near 100% or saturation. (Saturation, or 100% RH, is the condition necessary for wet-heat sterilization.)

The dry-heat death rate of a microorganism or a spore is related to the amount of water in the cell. It is also a function of the heating system. Microorganisms on the surface of a potato heated in an air oven is an "open system." If heating is carried out in an open system (8) the quantity of water in the cell or spore depends upon environmental factors such as (a) the initial water content of the microorganism or spore, (b) the type and density of the microbial or spore population, (c) the nature and relative humidity of the gaseous environment in contact with the microorganism, (d) the length of the heating period, and (e) the cleanliness of the deposit (7).

Angelotti et al. (1) studied the effect of spore moisture content on the dry-heat resistance of a spore population and found that maximum dry-heat resistance (largest D-value) occurred at a water activity (a_w) of 0.2 to 0.4. The D-value decreased at a_w levels below 0.2 or above 0.4.

Previous investigations in this laboratory (10) utilizing the planchet-boat-hotplate dry heat test method showed that the time required to produce a specific reduction in the number of surviving organisms varied with relative humidity. At 90 C, the time for a two-log reduction in the number of survivors was 53 h at 1.5% RH, 300 h at 15% RH, 190 h at 35% RH, 65 h at 55% RH, and 8.5 h at 75% RH. (RH values at 90 C.)

Similar conclusions were reached in studies to determine the effect of conditioning and treatment humidities on the dry-heat resistance of a spore population (3). Reducing the humidity level of either the

conditioning or treatment processes lowered the D-value of *Bacillus subtilis* var. *niger* spores on stainless steel surfaces. The suggestion was made that objects to be dry-heat sterilized should be conditioned in a low humidity environment to reduce the dry-heat process requirements.

The objectives of this study were (a) to determine the destruction characteristics of microorganisms on the surface of noninoculated heated and unheated potatoes and (b) to compare the dry heat destruction characteristics of *B. subtilis* var. *niger* spores deposited on metal surfaces with the results obtained when similar spores were deposited on potato surfaces, the latter were heated under conditions of time and temperature similar to those necessary to bake potatoes in a hot air oven as is done in the American home in preparing the food item "baked potatoes."

MATERIALS AND METHODS

Heating system

A Lab-Line Series 3810A "Reach In" High Temperature Oven with forced air circulation and a Partlow Temperature Control model RFC-15 was used throughout the heating study. The oven temperature control was set at 175 C but the temperature fluctuated between 173 and 175 C during the potato heating studies. A temperature of 175 C (347 F) was chosen arbitrarily as being within the range of temperatures commonly used for preparing homebaked potatoes (4). The oven was operated at temperatures of 125, 140, 155, and 175 C in the studies to determine the heat destruction characteristics of *B. subtilis* var. *niger* spores in tin cups.

Temperature measurement

The experimental temperatures utilized during the heating studies were sensed by copper-constantan thermocouples. A system of five copper-constantan thermocouples was used to monitor the actual oven temperature (AOT), the external potato surface temperature (EST), internal temperature of the potato (IT), and the temperature of the tin cup (TTC). In potato heating experiments the AOT, EST, and IT were recorded every minute. In the tin cup heating experiments AOT and TTC were recorded every 6 sec.

The AOT was measured using two thermocouples. These two thermocouples were soldered directly onto wires connected to the racks in the oven. One was located above and the other below the test samples.

Thermocouples measured the EST and IT of a representative potato during each heating experiment. The IT measuring thermocouple was placed into a hole made by a 20-gauge hypodermic needle extending half-way through the potato. The EST thermocouple was prepared by removing 5 inches of insulation at the terminal end of the thermocouple wire. An insulated Solderless Butt Connector model #357302 (Vaso Products, Inc., Chicago, Ill.) was positioned on the separated strands of wire before the ends were soldered together. The two separated strands were then shaped into a circle and the bare wire ends were soldered together against a flat surface. At time of use the circular wire was placed around the perimeter of the potato and the Solderless Butt Connector was moved down to the potato surface to keep the wire taut around the potato and the thermocouple in contact with the potato surface.

To develop an EST and IT temperature curve for an average potato during a routine heating experiment, the temperatures of eight potatoes were monitored for their individual EST and IT during heating at 175 C AOT. Individual heating curves for each of the eight potatoes were constructed. Temperature values for the eight potatoes at each respective time point were averaged and the values plotted to yield a curve representative of the time-temperature condition on the potato surface during the baking process.

To measure the TTC during heating, a tin cup was soldered to the

internal flat bottom surface of a Thermal Death Time (TDT) Can and a thermocouple soldered within the well to the cup bottom. The thermocouple was constructed with long, durable extension wires that made it possible to remove the TDT unit measuring TTC from the oven along with the test TDT unit without interrupting temperature measurement.

Spores

B. subtilis var. *niger* (University of Minnesota Environmental Microbiology Laboratory code AAEF) was used in all spore survival experiments. The spores were grown in our laboratory from spores supplied by the Communicable Disease Center Field Station, Phoenix, Arizona. The spores were grown in Synthetic Sporulation Medium 10 (5) in mechanically shaken flasks at 32 C for 48 h. The spore population was washed, transferred to sterile screw cap test tubes, and stored in distilled water at approximately 4 C. The spore suspension titer was 1.1×10^8 spores per ml.

Spore carrier test surfaces

B. subtilis var. *niger* spores were deposited on two types of spore carriers; (a) skin of washed potatoes, and (b) sterile tin-plated cups (11 mm dia., 8.5 mm deep). All inoculation and recovery procedures were performed in a Class 100 laminar downflow clean room operating at 22 C and 50% relative humidity. The operational regimen offered minimal opportunities for contamination.

Washing of potatoes by insonation before deposition of spores

Potatoes used in spore survival experiments were cleaned using insonation. Each potato was transferred to a sterile 600-ml Pyrex beaker; 200 ml of sterile phosphate (pH 7.2) plating buffer (SPB), was added to the beaker and the beaker containing the potato placed in the ultrasonic tank. Following insonation for 2 min, the potato was removed from the beaker and aseptically hand-rubbed while a 50-ml SPB rinse was poured over the surface. Washed potatoes were placed on sterile stainless steel trays and allowed to equilibrate overnight in the clean room before the spore deposition was made. Washed potatoes were titered for zero time normal flora population numbers.

Spore recovery

A Sonogen-A ultrasonic tank (Branson Instruments, Inc., Stamford, Conn.) operating at a frequency of 25 KHz/sec was used to remove the spores from the test surfaces. The position of the Pyrex container holding either the potato or the tin cup was adjusted so that the level of buffer in the container was the same as the level of fluid in the tank. The aqueous tank fluid contained 0.3% by volume of Tween 80.

Potatoes

Red River Grade "B" potatoes purchased from a local distributor (Kruger, Inc., St. Paul, MN) were used in these studies. All potatoes were from the same crop and had undergone similar harvesting and storage procedures before acquisition. The potatoes were small in size; the diameters ranged from 4 to 5 cm. Potatoes were stored in a walk-in refrigerator held at 4 C (Lab-Line Environments, Inc., Melrose Park, Ill.) Potatoes were handled aseptically under all circumstances using sterile rubber gloves so as not to contaminate the surface of washed, unwashed, heated, or unheated potatoes.

Deposition of spores

Each spore deposit, regardless of test surface, was made using an Eppendorf Push Button Pipet (Brinkman Instruments, Westbury, NY) having a delivery capacity of 20 μ l.

A test tube of spores was removed from the 4-C refrigerator and agitated using a vortex mixer for 15 sec to insure a uniform suspension. Between depositions the suspension was agitated to prevent the spores from settling. The spores were aseptically transferred from the test tube to the test surface using the Eppendorf pipette. Before starting, the Eppendorf was rapidly filled and emptied a few times to prevent bubble formation in the 20- μ l tip. In tests involving the tin-plated cups, the 20- μ l spore suspension was delivered directly into the well of the cup. In potato studies the spore deposition was made on a relatively flat area of potato. Following spore deposition, both potatoes and cups were equilibrated in the clean room at 22 C and 50% relative humidity for 18

to 24 h. During this period a stainless steel tray was placed over the inoculated surfaces to protect the surface from the direct air currents in the clean room.

Potato testing program

All potatoes involved in a single test were placed on the oven rack at the start of the experiment. The zero time samples were transferred to sterile, foil-covered, 600-ml Pyrex beakers and refrigerated at 4 C awaiting spore recovery. At the end of each heating time the oven doors were opened and two potatoes (in one experiment three potatoes were removed at each sampling time) were aseptically withdrawn, transferred to sterile, foil-covered, 600-ml Pyrex beakers, and refrigerated at 4 C.

Handling and spore recovery procedures for normal flora experiments were identical with those used in inoculated potato experiments. Aseptic precautions were followed in handling all of the potatoes. Potatoes utilized for obtaining the normal flora thermal resistance pattern and those for spore-inoculated thermal resistance patterns were never heated together.

In all analyses, 200 ml of SPB was added to the 600-ml beaker containing the potato. This volume was enough to fully immerse the potato during the 2-min insonation period. Aliquots of this rinse fluid were plated.

Procedures used to recover the normal flora of the potato skin were identical to procedures used to recover organisms from the inoculated potatoes.

Heating spores in tin cups

After 18 to 24 h of drying time the inoculated tin cups were placed in sterile thermal death time (TDT) cans (61 mm in diameter × 9 mm in depth) with removable lids. Two tin cups in a TDT can constituted a TDT unit. An 18-inch rubber-tipped crucible tong was used to insert the TDT unit into and remove it from the oven. Using the tongs, the lid was removed from the can after it had been inserted into the oven. Since heating times were short, TDT units were heated individually.

The TDT unit was rapidly placed in the oven and the lid was removed. The oven doors were closed and remained shut for the allotted heating time and were reopened immediately upon completion of the heating period. The lid was replaced and the TDT unit was removed from the oven with the 18-inch crucible tongs.

Each time the oven doors were opened at the end of a heating time, there was a concomitant depression in the AOT. The oven was allowed to re-equilibrate to the test temperature before starting the next heating interval.

Spore recovery began immediately upon the completion of the heating treatments. TDT units were opened and the individual cups were transferred to sterile 125-ml Erlenmeyer flasks using sterile forceps. A volume of 100 ml of SPB was added to the 125-ml Erlenmeyer flask containing a tin cup. The flask was suspended in the center of the ultrasonic tank for insonation. Each cup stood upright in its buffer (bottom side down) during the 2-min sonication treatment.

Plating procedure

All recovery procedures for spores on potatoes and in TDT units were carried out in the clean room. Trypticase Soy agar (TSA, BBL) was used as the recovery medium. SPB was used in all experimental dilutions. Dilution blanks consisted of 100 ml of SPB. For each sample the appropriate 0.1, 1.0, or 10-ml aliquot of the SPB suspending buffer was plated in duplicate. When 10.0-ml aliquots were plated, 1.5 strength TSA was used. Plates were incubated at 32 C for 48 h and then counted.

Treatment of data

The equivalent heating time (U) assuming instant heating and cooling was determined (9) so a semilogarithmic survivor curve (logarithm of the number of survivors vs. U) could be plotted and the D-value determined.

The D-value (time for a 90% reduction in the microbial population) for each heating experiment was calculated as the negative reciprocal of the slope of the regression line of the semilogarithmic survivor curve. The survivor data for unheated controls (N_0) were not used in the

D-value determination. The zero time intercept of the regression line (Y_0) was calculated. The Y_0 and N_0 were used to calculate the intercept ratio:

$$IR = \log Y_0 / \log N_0$$

The z-value was determined analytically by a least squares regression analysis of the logarithm of the D-values as a function of temperature. The z-value is the degrees of temperature change necessary to produce a 10-fold change in the D-value.

RESULTS

Heat resistance of spores in metal cups.

The time-temperature data for the thermal death time cups in open cans were plotted on semilogarithmic paper according to the method of Ball (2). Straight line heat penetration curves were obtained at all temperatures. The average f_h -value was 1.06 min, the average j-value was 1.7.

The D and IR-value data for spores heated in cups are listed in Table 1.

TABLE 1. Heat resistance of *Bacillus subtilis* var. *niger* spores in tin plated cups heated in an air oven

Temperature (C)	D-Value (min)	Intercept ratio ^a
125	14.1	1.07
125	10.3	1.05
140	2.9	1.00
155	0.656	0.96
175	0.065	0.79
175	0.048	0.79
175	0.077	0.76

^aIntercept Ratio [IR] — $\log Y_0 / \log N_0$

The D-value data in Table 1 were fitted to the Bigelow thermal death time curve model and the z-value was found to be 21.8 C; the 95% confidence limits were 19.9 and 24.0 C. The D(150 C) value was found to be 0.92 min.

The data in Table 1 show a consistent decrease in the intercept ratio with increasing temperatures. At 125 C the survivor curves were concave downward, however, at 175 C the survivor curves were concave upward.

Heat resistance of microorganisms on potato surfaces

Typical arithmetic temperature curves for potatoes heated in the hot air oven are shown in Fig. 1. The oven temperature varied cyclically over a 2-C range. The potato surface temperature also fluctuated 2 to 3 C. The AOT decreased rapidly when the oven doors were opened to remove potatoes.

The normal flora population values of unheated potatoes are listed in Table 2 and are approximately 10^8 CFU per potato. "Larger" potatoes did not exhibit higher microflora counts compared to "smaller" potatoes. Washed potato surfaces yielded fewer recoverable organisms compared to unwashed potato surfaces.

The numbers of aerobic microorganisms recovered from non-inoculated potatoes heated in a 175 C oven are shown in Table 3; both the heating time (total time in the oven) and the equivalent minutes are shown. A graph of the log of the numbers of survivors as a function of

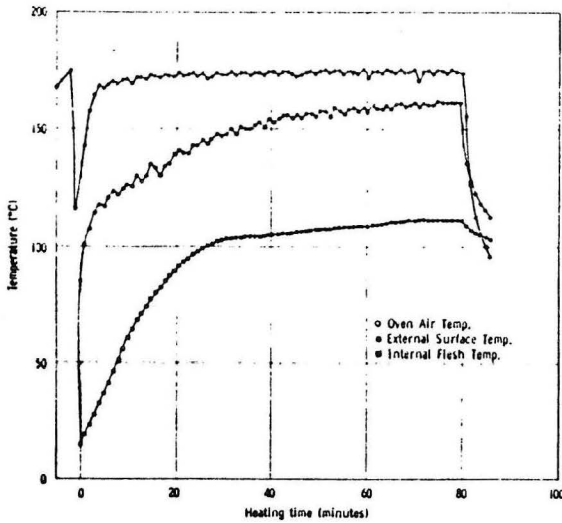


Figure 1. Internal and surface temperature of potatoes heated in an air oven at 175 C

TABLE 2. Aerobic microflora recovered from uninoculated and unheated potato surfaces

Experiment number	Population numbers (CFU/potato) ^a
Unwashed potatoes	
1	2.5 × 10 ⁸
2	3.69 × 10 ⁸
3	7.85 × 10 ⁷
Washed potatoes	
4	3.16 × 10 ⁷
5	4.5 × 10 ⁷

^aColony-forming units per potato

TABLE 3. Aerobic microflora recovered from uninoculated potatoes heated at 175 C

Heating time in 175 C oven (min)	U Equivalent min at 150 C	Population numbers (CFU/potato) ^a	
		A	B
0	0	3.7 × 10 ⁸	7.8 × 10 ⁷
5	0.01	6.0 × 10 ⁷	—
10	0.04	4.2 × 10 ⁷	4.6 × 10 ⁷
20	0.26	3.6 × 10 ⁶	1.2 × 10 ⁷
30	0.90	3.0 × 10 ⁵	9.7 × 10 ⁵

^aEach value, colony-forming units per potato, is the log average of duplicate samples.

clock-heating time (instead of equivalent minutes) is shown in Fig. 2.

The data in Table 3 were subjected to a semilogarithmic curve analysis (logarithm of number of survivors vs. equivalent heating time) and the D- and IR- values determined. In experiment A the D(150 C)-value was 0.40 min, IR was 0.89; in experiment B the D(150 C)-value was 0.53 min, IR was 0.97.

The number of *B. subtilis* var. *niger* spores recovered from inoculated potatoes heated in the air oven at 175 C are shown in Table 4. A graph of the logarithm of the number of survivors as a function of heating time is shown in Fig. 3. The data indicate that at least for the first 60 min of heating the logarithm of the population decreased with heating time. It must be kept in mind that in Fig. 2 and 3 are shown clock-heating time and not

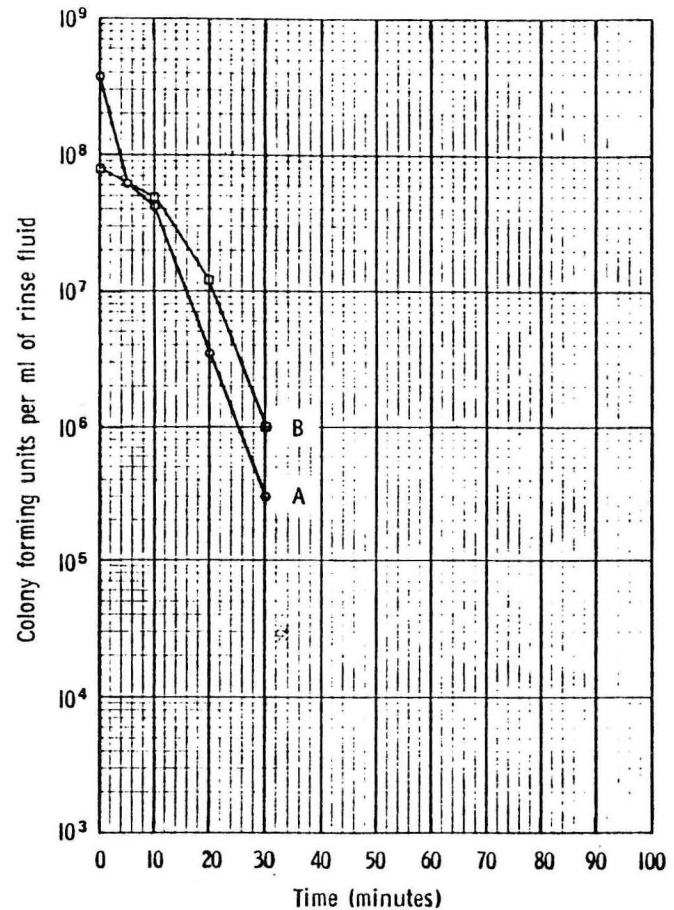


Figure 2. Semilogarithmic survivor curve for normal flora recovered from potatoes heated at 175 C.

equivalent-time.

The data in Table 4 were subjected to a semilogarithmic survivor curve analysis and the D- and IR-values determined. The results are shown in Table 5. The average D(150 C)-value, considering only the data for up to 60 min of heating, was 5.6 min.

TABLE 4. *Bacillus subtilis* var. *niger* spores recovered from potatoes heated at 175 C

Heating time in 175 C oven (min)	U Equivalent min at 150 C	Population numbers (CFU/potato) ^a		
		A	B	C
0	0	3.1 × 10 ⁷	5.5 × 10 ⁷	2.1 × 10 ⁷
10	0.04	2.0 × 10 ⁷	1.8 × 10 ⁷	1.0 × 10 ⁷
25	0.50	8.1 × 10 ⁶	1.2 × 10 ⁷	3.4 × 10 ⁶
45	3.33	3.2 × 10 ⁶	2.3 × 10 ⁶	4.6 × 10 ⁵
60	7.53	1.6 × 10 ⁶	3.5 × 10 ⁶	4.6 × 10 ⁵
70	11.20	—	1.7 × 10 ⁴	—
80	16.80	—	1.8 × 10 ⁴	—
90	25.10	1.0 × 10 ³	—	—

^aResults for experiments A and B are the log averages of duplicate samples; for experiment C the log averages of triplicate samples.

DISCUSSION

Results of this study indicate that similar populations of *B. subtilis* var. *niger* spores on tin-plate and potato surfaces tested under dry heat conditions have different survivor rates. The D(150 C)-value was of the order of 5.6 min (considering 60 min of heating) for the spores on

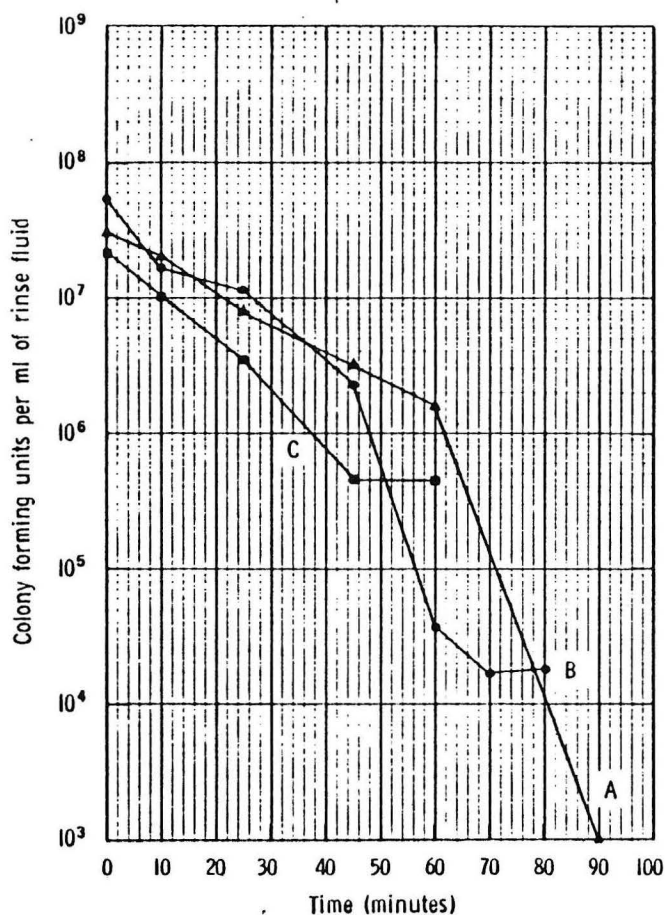


Figure 3. Semilogarithmic survivor curve for *Bacillus subtilis* var. *niger* spores recovered from potatoes heated at 175 C.

TABLE 5. Heat resistance of *Bacillus subtilis* var. *niger* spores on potatoes heated in an air oven at 175 C^a

Experiment	Heating time in 175 C oven (min)	Analysis based on total heating time		Analysis based on 60-min heating time	
		D(50 C) (min)	IR	D(150 C) (min)	IR
A	90	6.1	0.96	7.8	0.95
B	80	5.0	0.89	2.8	0.95
C	60			6.1	0.91
		$\bar{x} = 5.6$			

^aTwo regression analyses were made in two of the experiments, the first with the data up to and including 60 minutes of heating and the other using all the data.

the potatoes compared to a calculated D(150 C) value of 0.92 min. for the *B. subtilis* spores on a metal surface.

The normal microflora on potatoes decreased more rapidly than *B. subtilis* var. *niger* spores which were deposited on potato surfaces. The D(150 C)-values of the normal microflora of the potato was found to be 0.40 to 0.53 min. The average D(150 C)-value for *B. subtilis* var. *niger* spores on potatoes was 5.6 min, based on 60 min of heating (Table 5).

The D-value for the *B. subtilis* var. *niger* spores on potatoes was about 10 times greater than the D-value of the normal potato microflora. The D-values for the *B. subtilis* var. *niger* spores on the potato surface were about five times greater than for the *B. subtilis* var. *niger*

spores on tin-plate surfaces.

The results of the comparison of D-values indicate that a potato surface was a much more hospitable environment for spores during a dry-heat treatment than was a tin-plate surface. The nature of the two surfaces was very different, one being a hard metallic surface, the other a rough porous organic surface. Undoubtedly, the characteristics of the surface have an effect on the spore survival rate; however, there are other major differences in these environments. The tin-plate surface was in water vapor equilibrium with the atmosphere in the air oven. In contrast, the spores on the surface of the potato can be assumed to be continuously at a higher vapor pressure than the atmosphere in the air oven since water will be continuously diffusing from the potato. The potato can be assumed to be surrounded by a thin film of gas that has a high relative humidity. The humidity in this film was produced by the moisture diffusing from the potato to the air in the oven.

Heating of the tin-plate surface to the temperature of the air in the oven was rapid and uneventful. In contrast, heating of the potato in the oven was slow and extremely complex. The rate of heat transfer from the air in the oven to the potato was relatively low because of the low surface film heat transfer coefficient. Since the potato has a relatively high heat capacity and also loses water through evaporation during the heating period, the rate of heat gain of the potato was relatively low. During the baking process the surface of the potato on which the microflora was located was actively involved in the heat transfer and moisture transfer processes and at the same time underwent a major change in water content as the baking process proceeded.

At the start of the baking process, the water content of this outside skin of the potato and probably the microorganisms on the surface of the potato were in equilibrium with a relative humidity of about 85% at ambient temperature conditions. At the end of a 60-min baking period, it was probable that the relative humidity below the surface of the potato was still about 85%. During the baking period, the outside layers of cells of the potato will have been reduced in moisture content and at the same time will have become increasingly impervious to the diffusion of water vapor from the potato. During this period the microorganisms on the potato surface were enclosed by a film formed by the water vapor that was diffusing from the potato to the air in the oven. The authors believe that the water diffusing from the potato surfaces effectively raises the ambient relative humidity around the spores on the surface of the potato, which in turn alters the amount of water inside the spore, which in turn greatly changes the D-value of the spore population.

Only bacterial spores will survive moderate dry heat treatments. Therefore, we can assume that the cells of pathogens such as *Salmonella*, *Shigella*, and *Staphylococcus*, if located on the surface of a potato, should be destroyed during baking.

Perkins (6) reports dry heat kill times for spores of a

number of pathogenic organisms, including *Clostridium botulinum*, *Clostridium tetani*, *Clostridium perfringens*, as well as *B. subtilis*. The *B. subtilis* spores were more resistant than any of the above-mentioned spores.

We found a D(150 C)-value of 5.6 min for *B. subtilis* var. *niger* spores on potato surfaces during baking for 60 min at 175 C. If we assume, based on the data of Perkins (6), that *B. subtilis* spores have two times the dry heat resistance of spores of *C. botulinum* and *C. perfringens*, then the D(150 C) for spores of *C. botulinum* and *C. perfringens* would be on the order of 2.5 to 3 min on potato surfaces. This suggests that for 60 min of heating at 175 C there would be about a 3-log reduction of spores of pathogenic organisms on a potato surface.

These results indicate that under certain conditions it may be possible that viable spores or pathogenic microorganisms are being ingested when baked potato skins are eaten. However, there is no epidemiological evidence that eating potato skins is hazardous.

CONCLUSIONS

The conclusions that can be drawn from this series of studies are: (a) The D-value of microorganisms present on the surface of a potato during the baking process will probably be an order of magnitude less than that of organisms on a metal surface. (b) The dry heat treatment received by the surface of a potato during baking at 175 C will not destroy all of the dry heat resistant spores on this surface.

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