Survival of Bacteria in Artificially Contaminated Frozen Meat Pies after Baking

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The tremendous growth of the frozen food industry during the past decade has resulted in the advancement of bacteriological standards for frozen foods by certain agencies. Some frozen food plants already have self-imposed bacteriological standards and quality control functions. To help complete the picture on the microbiology of frozen foods before bacteriological standards are imposed, information should be acquired regarding the survival of bacteria in precooked frozen foods after baking.

There have been several reports in the literature concerning the numbers and the species of bacteria which were encountered in unbaked frozen meat pies by Larkin, Litsky, and Fuller (1955), Canale and Ordal (1957), Litsky, Fagerson, and Fellers (1957), Huber, Zaborowski, and Rayman (1958), Ross and Thatcher (1958), and Kereluk and Gunderson (1959). However, there is little information concerning the effect of baking on the microbial flora of frozen meat pies.

Studies by Canale and Ordal (1957) on frozen poultry pies have indicated that some of the times and temperatures recommended by the manufacturers are not sufficient to eliminate the nonsporeforming organism. It was also found that the center of the pies baked, under what the authors assumed to be home conditions, did not reach a temperature high enough to reduce the bacterial count satisfactorily. Coliforms, as well as other nonsporeformers, were detected in the baked pies.

Ross and Thatcher (1958) in a study of the bacteriological content of marketed precooked frozen foods in relation to possible public health hazard have shown that there is a 57.5 to 100 per cent reduction in the number of organisms found by the standard plate count after baking. However, the data presented on the effect of baking on the microbial population was limited to only a determination of the total number of bacteria.

The purpose of this investigation was to determine the effect of baking on the number of viable bacteria of sanitary and/or public health significance and on the numbers of sporeforming organisms artificially introduced into chicken meat pies.

Materials and Methods

The descriptions of the bacterial cultures used in this investigation are as follows. Escherichia coli was isolated from a high count beef dinner cultured on deoxycholate lactose agar. The organism was characterized as follows: gram negative, nonsporeforming rod; lactose fermenting with gas in 24 hr; indole and methyl red positive, Voges-Proskauer and citrate negative. The organism gave typical reactions on differential media. Staphylococcus aureus was isolated in pure culture from brand beef dinner cultured on a salts agar plate. The organism had the following characteristics: gram positive, spherical cells in grape-like clusters; colony exhibited a golden yellow pigment; β-hemolysis on blood agar plates; and gelatinase and coagulase positive. Bacillus subtilis was isolated from a trypotone glucose extract agar plate run on a high count chicken meat pie and demonstrated the following characteristics: gram positive, aerobic, sporeforming, long rod occurring in long chains; and liquefied gelatin. The isolation of Streptococcus faecalis was made from a positive ethyl violet azide broth tube. A streak was made on a trypotone glucose extract agar plate and an individual colony was picked and transferred into brain heart infusion broth. The organism was a gram positive coccus, occurring in short chains; lacked any hemolytic properties, met Sherman's (1937) criteria by growing in a broth at a pH of 9.6 in broth with 6.5 per cent sodium chloride, in skim milk with 0.1 per cent methylene blue, and in broth incubated at 45 C, and gelatinase negative.

The artificially inoculated chicken meat pies were made in the laboratory. The organisms were seeded into separate aliquots of 100 ml of brain heart infusion broth and incubated for 24 hr at 37 C. Fifty milliliters of each culture were transferred into separate containers of sterile chicken gravy from which the chicken meat pies were made. A total count of the organism in the gravy was made before the pies were frozen. The pies were held in a wind tunnel at −40 C for 6 hr and then stored in a laboratory freezer at −13 C until used in this study.

1 Presented at the 87th Annual Meeting of the American Public Health Association, Laboratory Section, Atlantic City, New Jersey, Wednesday, October 21, 1959.

2 Present address: Senior Bio Scientist, Space Medicine, Boeing Aircraft Company, Seattle, Washington.
All the chicken meat pies were first sampled by gently removing a portion of the outer crust with a sterile spoon and removing an 11-g gravy sample which was aseptically transferred into a 90 ml dilution blank with 0.1 per cent peptone water. Because the bacteria were present in the gravy, only the gravy was sampled for bacteriological enumerations. The frozen chicken meat pies were placed into a gas oven preheated to 218 C (425 F). A series of pies were baked at each of three different time periods, 20, 30, and 40 min. The pies were sampled for bacterial numbers immediately after baking, 10 min after baking, and 20 min after baking. The determinations for each type of the organism were made from the pie samples as follows. The most probable number method (MPN) and an agar plating method using deoxycholate lactose agar were used to determine the numbers of *E. coli*. The MPN method consisted of five replicate tubes of serial dilution in lactose broth. If there was evidence of gas in the lactose broth tubes, transfers of 0.1 ml were made into brilliant green bile broth tubes for confirmation. At the end of 24 hr, the brilliant green bile broth tubes were read for gas production. Gas production in the brilliant green bile broth tubes denoted a positive test. For the plating method, serial dilutions of the sample were plated using deoxycholate lactose agar. The plates were incubated for 24 hr at 37 C. Mannitol salts agar was used as the plating medium for the enumeration of *S. aureus*. Only colonies demonstrating a yellow zone were counted and considered to be the organism. The plates were incubated at 37 C for 48 hr. The MPN method, as described by Litisky, Mallmann, and Fifield (1953), was used for the enumeration of *S. faecalis*. The MPN was obtained by adding 10-, 1-, 0.1-, and 0.001-ml portions of the sample to sets of five replicate azide dextrose broth tubes (AD). After 48 hr incubation at 35 C, 0.1 ml-samples from the positive AD tubes were transferred into ethyl violet azide broth tubes (EVA) for confirmation. The EVA tubes were incubated for 48 hr at 37 C. Turbidity and the formation of a purple button on the bottom of the EVA broth tubes were considered to be a positive confirmatory test. For the enumeration of *B. subtilis*, tryptone glucose extract agar was used as the plating medium. The plates were incubated at 37 C and counted after 48 hr.

A series of heat penetration investigations were made using an electronic recording potentiometer and a ceramic, copper-constantan thermocouple. Using an electric drill with a \( \frac{3}{4} \) in. bit, a hole was bored into the center and half way down into the frozen meat pie. The hole was filled with gravy and then the ceramic thermocouple was inserted into the hole and secured in place on an aluminum tray by strapping to a metal frame. The pie was placed in a freezer for a period of \( 2\frac{1}{2} \) hr to freeze the added gravy around the thermocouple. The product with the thermocouple was removed from the freezer, the thermocouple connected to the electronic recording potentiometer, and the pie placed into a gas oven preheated to 218.8 C (425 F) and baked. Temperatures of the oven were also recorded.

### RESULTS AND DISCUSSION

Determinations were made on the effect of freezing on the total numbers of bacteria present in the inoculated gravy in chicken meat pies. The drop in total number of organisms was so small as to warrant dropping the determination of the number of bacteria after freezing from the bacteriological procedure of the laboratory made pies. The mass inoculation of bacteria

<table>
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<tr>
<td>30</td>
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<tr>
<td>40</td>
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* An average determination of 24 pies.

### TABLE 1

*Temperatures attained in the center of artificially inoculated chicken meat pies during baking*

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<th>Temperature*</th>
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<tr>
<td>40</td>
<td>214.0</td>
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### TABLE 2

*Effect of various baking periods at 425 F on the survival of *Escherichia coli* in chicken meat pies*

<table>
<thead>
<tr>
<th>Before Baking</th>
<th>Bacteriological Sampling Immediately after Baking</th>
<th>Bacteriological Sampling 10 Min after Baking</th>
<th>Bacteriological Sampling 20 Min after Baking</th>
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</table>

* Figures are number of bacteria per gram as determined by the MPN method.
in the pies before baking was to ensure large population to determine the possible lethal effect of the various baking periods. It has been our experience (Kereluk and Gunderson, 1959) that the majority of meat pies obtained at the retail outlets are of good bacteriological quality and this necessitated artificially contaminating meat pies to obtain the level of bacterial numbers needed in these experiments.

In determining the total number of E. coli in the inoculated pies, two methods were used, a plating method using deoxycholate agar and the method of MPN. The results from the two methods varied greatly. Therefore, the plating method for the determinations of total numbers of E. coli was discontinued.

The heat penetration investigations revealed an average heat penetration rate of 4.5 F per min, when the pies were baked in a gas oven at 425 F for 40 min (table 1). The internal temperatures attained in the center of the meat pies at the different baking times were: 124.0 F for a 20-min baking period, 170 F for the 30-min baking period, and 214 F for the full 40-min baking period. The internal temperatures were determined from an average of 24 pies at each of the different baking times.

| TABLE 3 |
| Effect of various baking periods at 425 F on the survival of Streptococcus faecalis in chicken meat pies* |

<table>
<thead>
<tr>
<th>Before Baking</th>
<th>Bacteriological Sampling Immediately after Baking</th>
<th>Bacteriological Sampling 10 Min after Baking</th>
<th>Bacteriological Sampling 20 Min after Baking</th>
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<table>
<thead>
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<th>Twenty-Minute Baking Period</th>
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<tr>
<td>130,000</td>
</tr>
<tr>
<td>11,000</td>
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</tbody>
</table>

* Figures are number of bacteria per gram as determined by the MPN method.

The effect of baking on E. coli in chicken meat pies is tabulated in table 2. The baking times of 40 and 30 min completely eliminated the organism. However, the 20-min baking period produced varied results with a slight reduction in the number of the organisms in the pie.

An examination of the results tabulated in table 3 indicates that there was a considerable drop in the number of S. faecalis in the 40-min baking period; however, there was one exception. In sample one, the original count of 460,000,000 bacteria per g had dropped to 290 bacteria per g after the 40-min baking period. The effect of 30 min of baking on S. faecalis was not as great as the 40-min baking period. There was a reduction in numbers of the organism by the end of the baking period and a further drop after the 10-min sampling period. After the 20-min baking, the results were varied and could not be evaluated.

The effect of the different baking times on S. aureus is tabulated in table 4. The results indicate that the original count of the organism was 1,000,000 to 5,000,000 organisms per g, but after the 40-min baking period, the count dropped to a range of 0 to 40 organisms per g. Ten minutes after the baking period, the counts ranged between 0 to 10 organisms per g. The 30-min baking period caused a decline in...
the total number of *S. aureus*. However, after the 10- and 20-min waiting periods after baking, the counts remained constant.

The numbers of *B. subtilis* before baking ranged from 800,000 to 40,000,000 organisms per g (table 5). After the 40-min baking period at 425 °F, the range was 2 to 80,000 organisms per g. After the 10-min hold period, the number of *B. subtilis* continued to drop with one exception. After the 30-min baking period, *B. subtilis* demonstrated a drop in numbers, and this trend continued after the 10-min hold period.

In the bacteriological examination of chicken meat pies, no determinations were made for spores since the inoculum used was an 18-hr culture of *B. subtilis* grown in brain heart infusion broth. It has been the authors' experience that in plant conditions spore-formers were usually present in greater numbers in the vegetative state than in the spore state in frozen foods. Survival of *B. subtilis* after baking was probably due to the presence of spores which may have developed during the prefreezing handling period and cannot be discounted. Further investigations are necessary to determine the survival of spores in frozen meat pies after baking.

**TABLE 5**

**Effect of various baking periods at 425 °F on the survival of *Bacillus subtilis* in chicken meat pies**

<table>
<thead>
<tr>
<th>Before Baking</th>
<th>Bacteriological Sampling Immediately after Baking</th>
<th>Bacteriological Sampling 10 Min after Baking</th>
<th>Bacteriological Sampling 20 Min after Baking</th>
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<tbody>
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<table>
<thead>
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<th>Thirty-Minute Baking Period</th>
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<table>
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</table>

* Figures are number of bacteria per gram as determined by the MPN method.

The pies that were baked for 20 min at 425 °F were unpalatable because the crust had just started to brown and the pies had the appearance of being just "thawed" or raw. It is highly unlikely that a pie baked for 20 min would be consumed. Despite the indicated fact that baking will greatly reduce the number of bacteria present in a "grossly contaminated" meat pie, frozen meat pies should be produced with the lowest possible number of bacteria and under the maximal amount of sanitation. Baking of frozen meat pies should not be relied upon to mask a poor sanitary operation.

**SUMMARY**

To determine the effect of various baking periods at 425 °F on the survival of various organisms, a series of chicken meat pies were inoculated with a heavy suspension of bacteria prior to freezing. The bacteria used in the investigation were *Staphylococcus aureus*, *Streptococcus faecalis*, *Escherichia coli*, and *Bacillus subtilis*, and only one species of bacteria was inoculated into each pie. The contaminated pies were baked for 20, 30, and 40 min in a gas oven preheated to 425 °F and the pies were sampled for bacteriological counts at the end of the baking periods and again at 10 and 20 min after baking.

There was a 0.0002 to 0.1 per cent survival of the nonsporeforming bacteria after baking at 425 °F for 40 min. In the pies inoculated with *S. faecalis*, the survival was 0.1 per cent. The sporeforming organism, *B. subtilis*, demonstrated a 0.35 per cent survival in the pies under these conditions.

In the pies baked for 30 min at 425 °F, there was a 0 to 3.6 per cent survival of the nonsporeforming organisms, *S. faecalis* again surviving in the greatest numbers.

After the 20-min baking period, the nonsporeforming bacteria exhibited a 14 to 100 per cent survival. The sporeforming bacterium had a survival of 150 per cent or more, indicating possible multiplication.

**REFERENCES**


Litisky, W., Fagerson, I. S., and Fellers, C. R. 1957 A
Effect of Irradiation on Growth and Enzyme Activity of *Aspergillus oryzae*

M. L. Fields, G. R. Ammerman, and N. W. Desrosier

Department of Horticulture, Purdue University, Agricultural Experiment Station, Lafayette, Indiana

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Beraha, Ramsey, Smith, and Wright (1959) showed that the medium upon which *Penicillium digitatum* was grown prior to irradiation had an effect upon the dosage needed to kill the mycelium. Information is needed, therefore, on the growth response of irradiated mycelium to explain reasons for growth response and survival in irradiated foods. This study was instituted to determine the effects of irradiation on the survival and enzyme activity of the mycelium of *Aspergillus oryzae*.

Materials and Methods

**Radiation facilities.** The radiation source was a linear accelerator built to operate between 2.0 and 6.0 MEV. The mycelium was irradiated in a glass chamber with two aluminum windows 1 mil in thickness. The cell was 2 cm thick and 3.8 cm in diameter. Irradiation was performed at 5.6 MEV. The energy levels of the electrons in the cathode beam varied 10 per cent. The beam was nonmonoeenergetic (MacKay, 1953). Dosimetry was performed using the cecic sulfate method (Weiss, 1952).

**Growth media.** Bacto-Czapek-Dox (sucrose-nitrate medium) and a starch broth medium were used both as a pre- and postirradiation growth medium. The starch medium was composed of the following (per L): soluble starch, 10 g, sodium nitrate, 3.0 g, dipotassium phosphate, 1.0 g, potassium chloride, 0.5 g, magnesium sulfate, 0.5 g, and ferrous sulfate, 0.01 g. The pH of the starch medium was adjusted to 5.5, which is optimum for the activity of α-amylase of *A. oryzae*.

**Mycelium production.** *A. oryzae* (Ahlburg) Collection strain 11601, was obtained from the American Type Culture Collection, Washington, D. C., and grown as a shake culture on both sucrose-nitrate broth and starch broth for 60 hr. At the end of this growth period, the mycelium was washed aseptically with sterile distilled water to remove all reducing sugars. The washings were tested for reducing sugars by the method of Sumner and Howell (1935). After washing, the mycelial pellets were ground in a Waring Blender to make a uniform suspension so that the mycelium could be pipetted aseptically into growth flasks.

**Radiation treatment.** The mycelium was irradiated over a range of 0.086 to 3.87 × 10⁴ rads in distilled water and placed in fresh sucrose-nitrate and starch-nitrate broth within 2 hr after irradiation.

**Growth measurement.** The presence or absence of growth was noted by visual observations. One milliliter of the irradiated mycelium was inoculated in 15 ml of the growth media. The samples were incubated at room temperature for a period of 5 days.

**Enzyme activity.** α-Amylase activity was estimated by adding an iodine solution (0.005 M iodine in 0.2 M potassium iodide). Decrease in the blue color of the starch medium as compared with the control (not inoculated with mold) was used as an index of α-amylase activity. Sucrase activity was measured by using the method of Sumner and Howell (1935). The presence of reducing sugars in the sucrose-nitrate medium was considered to be an indication of sucrase activity.

Results

When the mycelium was grown in sucrose-nitrate medium prior to irradiation, *A. oryzae* was not able to grow after having been exposed to a dosage of 0.43 ×