Ride into the Danger Zone:
The Effect of Time on the Rate of Bacteria Growth on Cooked Chicken Breasts at Room Temperature

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Safety & Sanitization

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Introduction

The Danger Zone is a noted hazard in food safety. According to USDA, “Bacteria grow most rapidly in the range of temperatures between 40°F and 140°F, doubling in number in as little as 20 minutes. This range of temperatures is often called the "Danger Zone."” (USDA, “Danger Zone”) It is important to note that even cooking food to a proper temperature does not kill all pathogens and bacteria that can cause foodborne illness (USDA, “How Temperatures Affect Food”). Leaving food at room temperature, instead of holding it correctly, can have disastrous results for those who eat it and those who serve it.

Fast food restaurant chain, Chipotle, came under fire in 2015 when around 50 people who dined at their establishments reported being diagnosed with E. coli afterward (Choi). Chipotle had always prided themselves on cooking food in house and using fresh ingredients. But it seemed that leaving all this cooking and preparation to minimally trained employees resulted in unsafe practices. As a result, the restaurant decided to make changes to ensure food would be cooked properly, held safely, and eventually the E. coli reports would stop.

Hypothesis

For my own experiment, I decided to see just how much time impacted food held at improper temperatures, otherwise known as the Danger Zone. I believe the bacterial growth that appeared after four hours of improper holding would rapidly increase, covering more than 40%

of the petri dishes. By six hours, the bacterial growth would cover more than 60%. Meanwhile, the control piece of chicken would show little to no bacterial growth at all, as it is going to be cooked thoroughly and held at the correct temperature.

Research

It is common knowledge that ill-prepared or kept foods can be dangerous. In 1993, over 700 people become sick, and four children died, when fast food chain Jack in the Box served its customers undercooked hamburgers (Vaida). This deadly tragedy served not only as an example of what happens when food safety is not observed, but it also resulted in making it illegal to sell hamburger meat infected with E. coli O157:H7.

In a professional kitchen, with all the demands and chaos of a mealtime rush, it can be easy to consider skirting around a safety rule. But short cuts in the kitchen can have truly disastrous results that will never justify saving a little time or effort.

As pointed out in Quantitative Microbiology: A Basis for Food Safety, predicting how microorganisms will grow in food is not a simple process. The number of variables, acidity of the food, time held, temperature held, fat content, etc., are so vast and complex that scientists who study these factors struggle to create reliable models (McMeekin, T.A., et al.). Which means your average cook in a restaurant has little to no chance of being able to spy a package of ground beef that has been sitting on the edge of their prep table for entirely too long and arcuately gauge whether it is still safe to prepare it for customers.

It is always in the best interest of everyone involved to not take the risk. Especially when those cooking most likely never interact with the customer. They have no idea if they are cooking for the most robust bodybuilder or someone with a weakened immune system.

One eye-opening experiment was The Effect of Time and Temperature Variations on the Microbial Load and Deterioration Criteria of Leftover Cheeseburger Sandwiches (Malak', N.M.L., and N.S.M. Soliman). This paper saw scientists collect 60 cheeseburgers from all over Cairo and Gaza and put them into three groups for testing. One group was kept refrigerated at 5°C (41°F), another was room temperature at 25°C (77°F), the last was kept warm, and well in the danger zone, at 37°C (98.6°F).

The scientists tested for multiple forms of bacteria and microorganisms during their experiment and they most certainly found them. Their table of results is just staggering. For example, a comparison of the cheeseburgers left to sit at 77°F:

Time	Staphylococci Count
30 minutes	3.55b,C±0.08
60 minutes	4.60b,B±0.09
120 minutes	5.85b,A±0.10

Figure 1: Cheeseburger Bacterial Count Comparison

It truly shows what a difference holding time alone makes when it comes to food safety. The bacterial growth is explosive. Also, the difference between 30 minutes, 60 minutes, and two hours is not even that great. For that many microorganisms to grow in that short period is sobering.

I believe I will see similar results in my experiment, as my research has the same nature.

Materials

The materials used for this experiment are simple:

- 8 petri dishes with lids
- 8 cotton-tipped swabs
- 4 chicken thighs, cut in half
- Digital thermometer

- 1-2 Tbsps. vegetable oil
- 7 small paper plates
- Plastic leftover dish with lid
- 15 printed cards denoting hold times
- Clear tape (I used packing tape)

Procedure

The experiment will be conducted as follows:

1. I will assemble all materials and begin by cutting the raw chicken thighs into similarly sized pieces, between 2.5 to 3 ounces each. For this experiment, I will need eight chicken thigh pieces total.
2. The chicken will then be cooked in the same pan with a little bit of oil until each piece reaches an internal cooking temperature of 165°F. This will be verified with a digital thermometer.

Figure 2: Chicken Reaches Temperature

3. The eight pieces will be each swabbed differently:

a. One piece will be swabbed immediately after cooking and reaching an internal temperature of 165°F
b. One will be held for one hour at room temperature
c. One will be held for two hours at room temperature
d. One will be held for three hours at room temperature
e. One will be held for four hours at room temperature
f. One will be held for five hours at room temperature
g. One will be held for six hours at room temperature
h. The last will be placed in the refrigerator after reaching an acceptable temperature to be held at 40°F for six hours and then swabbed (this will be the control)

Figure 3: Cooked Chicken Waiting to be Swabbed

4. Each piece will clearly marked with a card stating its holding time. The chicken held at room temperature will be held in a cold oven with the door cracked enough to allow air to circulate, but also to prevent the cats from becoming part of the experiment.

Figure 4: An Aforementioned Cat, Waiting for a Piece of Chicken

5. When swabbing the chicken, I will cut into the thickest part of the sample and swab each side of the cut. To apply the sample to the petri dish, I will use the zigzag streak method. During processing, I will also use a digital thermometer to gauge the chicken piece's internal temperature.
6. The petri dishes will be taped shut immediately after being swabbed. I will evaluate the results in 5 days, as recommended by the instructions that came with the dishes.

Observation and Results

When I first started preparing for the experiment, I realized the chicken thighs I obtained from the grocery store were coated in a lemon pepper sauce. Because the sauce was bound to be messy and served no practical purpose as the chicken was not going to be consumed, I quickly rinsed it off. After all, I was going to cut into the chicken and swab the meat in the cut, so washing the outside should not influence the experiment.

During the cooking and holding part of the experiment, the chicken retained its regular appearance. Which I found rather frustrating as I knew, especially towards the ends of the experiment, that the chicken was not safe to eat, even if it did not look like it. Each piece of chicken was swabbed on time and the dishes were taped shut to be read a few days later.

As I swabbed, I collected the temperatures of the chicken pieces. The chart below details what temperature each piece was right before I took the sample.

Temperature Tracker	
Held 1 Hour	**Held 2 Hours**
93°	75°
Held 3 Hours	**Held 4 Hours**
76°	75°
Held 5 Hours	**Held 6 Hours**
74°	73°
Held in Fridge	**Freshly Cooked**
40°	165°

Figure 5: Temperature Tracker

Five days later, I returned to petri dishes to read the results. Unfortunately, something had gone wrong. The plates had stubbornly refused to grow much at all.

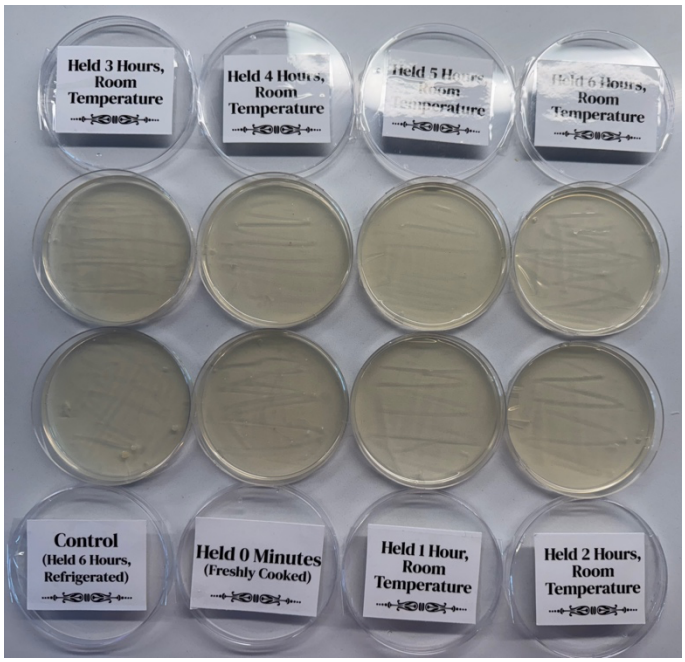

Figure 7: The Results

The only plate that had anything going on was the control, which had been refrigerated for six hours. Which is the one I figured would show the least amount of growth.

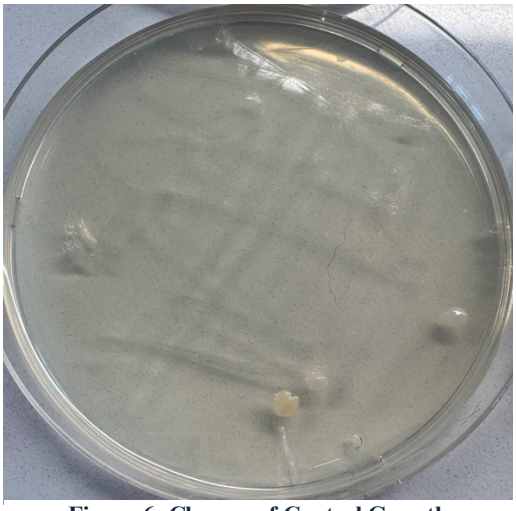

Figure 6: Closeup of Control Growth

This is all that grew out of all eight dishes. The upper left blotch is a tear in the agar and not a bacteria colony at all.

After much thought and disappointment that I would not get to see my glorious bacteria colony, I tried to figure out where it all went wrong. I came up with a few ideas.

1. **Rinsing the Chicken.** Although I had not given it much thought at the time. I wondered if rinsing the lemon pepper sauce off the chicken could have impacted the results of the experiment.
2. **User Error**. One of the reasons I was so excited to do this project was because I literally have not conducted a science experiment since grade school. Which was over 20 years ago. The chances I made a mistake while handling the chicken or the dishes, and did not even realize it, are probably higher than I am willing to admit.
3. **The Petri Dishes**. Out of curiosity, I decided to see what other people online were saying about the same petri dishes I purchased. While there are a lot of glowing reviews of these dishes, there are also over 100 negative ones. Many saying they had experiment results like mine, where nothing grew at all. Due to the low price and ease where I was able to obtain them, that could have been the issue.
4. **The Temperature in the House**. The petri dishes were supposed to have best developed under a heat lamp between 85°-100°F. Unfortunately, I did not have a heat lamp. Our home is around 70-72°F normally. Even though I waited the suggested number of days for the results to appear without the lamp, I wonder if it would have helped if I would have obtained a heat lamp to speed the process along.
5. **My Kitchen is just Entirely too Clean**. There were simply no bacteria to find the chicken to grow in the dishes. Right? While I would like to claim the issue this one, I think this is the least likely.

While I may never know what came between me and bacteria colony glory, I believe it may be down to a mixture of potential issues.

Conclusion

I am of two minds regarding this experiment. First, I knew I was not doing new research in the realm of food science to prove something novel. Whatever would I proven in my kitchen was going to be in line with what science has told us already through testing, research, and even more testing. It was not what I was proving that was the point, it was process and method to prove it.

At the same time, I ended up not proving anything. Except, perhaps, be more careful where you purchase science experiment supplies. Regardless of what went wrong, and maybe *because* it went wrong, I ended up very much thinking about the experiment, the process, the logistics of how science moves from hypothesis and planning to execution to analysis.

Do I know that leaving cooked chicken on the counter for six hours is a bad idea? Of course. I knew that before I ever started. It does not matter that I was not breaking new ground in science; I was breaking new ground for myself as a food scientist.

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