Validation of Baking to Reduce Coliform Counts and *Salmonella* in Cookie Dough

by
Julia Wilson

A THESIS

submitted to

Oregon State University

Honors College

in partial fulfillment of
the requirements for the
degree of

Honors Baccalaureate of Science in Food Science and Technology
(Honors Scholar)

Presented June 1, 2018
Commencement June 2018

AN ABSTRACT OF THE THESIS OF

Julia Wilson for the degree of Honors Baccalaureate of Science in Food Science and Technology presented on June 1, 2018. Title:
Validation of Baking to Reduce Coliform Counts and *Salmonella* in Cookie Dough

Abstract approved:_____

Joy Waite-Cusic

This study aimed to determine the efficacy of baking to reduce bacterial populations (an unknown contaminant or *Salmonella*) in cookie dough. Baking conditions (time and temperature parameters) were selected based on conditions commonly used in industry. The first study (Chapter 2), "Baking as Validation for Control of Coliform Counts in Cookie Dough", focuses on baking as means of controlling high plate counts found in cookie dough supplied by an industry partner. The second study (Chapter 3), "Validation of Baking as a Kill-Step for Salmonella and D-Value Determination for Inoculated Ingredients in Cookie Dough", validates baking as a kill-step to reduce *Salmonella* populations. In addition, this study examined whether the efficacy of baking was dependent on the contaminated ingredient. These studies demonstrated that baking is an effective lethal treatment to reduce vegetative cells (natural microbiota and *Salmonella*), but has minimal impact on spore populations. Baking effectively reduced *Salmonella* in cookies that made from contaminated flour, butter, eggs, and peanut butter; however, baking was less effective at inactivating *Salmonella* in contaminated chocolate.

Key Words: Lethal Treatments, Kill-step, Baking, Salmonella, Food Safety, Cookies, Chocolate

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APPROVED:

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I understand that my project will become part of the permanent collection of Oregon State University, Honors College. My signature below authorizes release of my project to any reader upon request.

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Julia Wilson, Author

Chapter 1. Introduction/Literature Review

Despite an increase in technology and awareness of food safety in the United States, foodborne outbreaks and consequential illnesses continue to exist. In 2016, the Center for Disease Control reported that annually, 48 million Americans get sick from foodborne illnesses. This results in approximately 128,000 hospitalizations and 3,000 deaths every year (CDC, 2018). Aside from personal suffering experienced by those affected by the unfortunate consequences of foodborne illnesses, the economy is also affected by a lack of safety in the food industry. According to a report published by the Produce Safety Project in 2010, a staggering $152 billion is estimated to be spent on foodborne illnesses every year in the United States (Scharff, 2010). Manufacturers are affected by foodborne illness because they must deal with recalls, which can cost companies millions of dollars to ensure consumer safety and rebuild consumer trust (Heneghan, 2016). In addition, this adds to the burden of food waste, which the Natural Resources Defense Council has estimated occurs with 40% of the food in America (NRDC). The damage that comes from foodborne illness affects personal lives and the economy in significantly negative ways. Clearly, this is an issue that demands serious attention and must be at the forefront of scientific research.

A significant portion of the American Diet comes from bakery products. For a 31 year old female, the United States Department of Agriculture recommends consuming 6 ounces of grain products per day (USDA, 2015). Many people consume these grains through processed products including bread, muffins, cookies, and granola bars because they are a convenient and inexpensive way to fulfill these daily requirements.

The bakery industry is responsible for producing massive amounts of the baked products that many Americans eat daily. According to Mordor Intelligence, the total market size of the bakery products is anticipated to reach USC 530 billion by 2023 (MI, 2017). With great influence comes great responsibility and the bakery industry needs to prioritize food safety. In the United States, 142 foodborne outbreaks and 2822 illnesses have been linked to bakery products between 2004 to 2013 (CSPI, 2015).

In the United States, the most common bacteria and viruses that cause illnesses, hospitalizations, and deaths are *Salmonella*, Norovirus, *Camplyobacter*, *E. coli*, *Listeria*, and *Clostridium perfringens* (USDHHS). ***Salmonella*** is a pathogen that can be found in the raw ingredients used to make the cookies and can survive low-water activity conditions for lengthy amounts of time (Podolak et al., 2010; Van Doren et al., 2013). ***Salmonella*** is estimated to cause 93.8 million cases of salmonellosis globally every year and 155,000 deaths occur annually because of ***Salmonella*** poisoning (Majowicz et al., 2010). One source of contamination in bakery products comes from eggs (USDA, 2016). However, many of ***Salmonellosis*** cases occur from ***Salmonella*** that came from low water activity products, such as flour, that are commonly used in bakery products. Contamination of ***Salmonella*** can happen because of ingredient contamination, inadequate sanitation procedures, poor maintenance, and facilities that do not meet standards (Carrasco et al., 2012). Because bakery products typically require a baking step, this is the primary method of lethal treatment for control of ***Salmonella*** in products such as bread, buns, muffins, and cookies. The objectives of this study were twofold: 1st) to validate a baking

process of 11 minutes at 163 °C for control of coliform counts in industry supplied cookie dough and 2nd) to validate a baking process of 11 minutes at 163°C to control *Salmonella,* determine whether or not initial ingredient inoculation in flour, egg, butter, or peanut butter affects kill rates, and to determine D-values (thermal inactivation parameters) for cookie dough made with either inoculated flour, egg, butter, or peanut butter.

Chapter 2. Baking as Validation for Control of Coliform Counts in Cookie Dough

Abstract

Coliform counts are a common indicator of unsanitary conditions in food processing facilities. A local food company has been having challenges with intermittent high levels of coliform counts in fully baked cookie product. They identified one ingredient, rice flour, as a potential source of coliforms in their ingredient list; however, the baking process was predicted to effectively reduce this population. This study sought to verify that the baking process would effectively reduce coliform counts in the cookie dough and assist the company with determining if their high counts were due to post-processing contamination. The local food company supplied cookie dough with high coliform counts for this study. Cookies were baked in a convection oven at 163°C for 11 minutes.Raw and baked cookie samples were serially diluted and plated onto TSA, where the colonies were counted after 24 hours. The microbial load of the raw cookie dough was 7.87 log CFU/gram whereas the microbial load of the baked cookie was 5.05 log CFU/gram. The results of the study indicated that the baking procedure controlled some of the bacteria, but did not completely eliminate it. Baking had little impact on the number of bacterial spores in cookie dough.

1. Introduction

A company that manufactures sandwich cookies for ice cream sandwiches had high coliform counts recorded from their finished products. Coliforms are not always harmful, but high coliform counts can indicate unsanitary conditions of water and food. Ice cream

sandwich cookies, usually composed of bleached flour, sugar, and palm oil have low water activities and do not support bacterial growth post-baking.

In order to reach their targeted coliform standards, the company needs to identify the source of their contamination and determine if the relative likelihood of coliform counts in the finished product is likely from survival during the baking process or cross-contamination following baking. To assist in this determination, we evaluated the efficacy of baking to reduce coliform counts in cookie dough supplied by our industry contact.

2. Methods

2.1 Cookie Dough Preparation

Cookie dough from the company was sent to the laboratory of Dr. Joy Waite-Cusic in the department of Food Science and Technology at Oregon State University and frozen at –18 °C until further use. The cookie dough was thawed in the refrigerator (4°C) one day in advance. On the day of baking, the cookie dough was scooped into 30-gram portions and formed into disks approximately 5.0 centimeters in diameter and 2.0 centimeters thick. Twelve cookies were weighed and placed on the cookie tray.

2.2 Cookie Baking and Temperature Measurement

The cookies were baked in a pre-heated 163 °C convection oven (Waring Commercial Half-Size Heavy-Duty Convection Oven, WCO500X, 1.5 cubic feet, Torrington, CT) for 11 minutes. Prior to baking, data logger thermocouples (Easylog EL-USB-TC, Lascar

Electronics, Erie, PA) were inserted into the cookie dough disks at the back, middle, and front of the oven to monitor baking temperatures. In addition, data loggers monitored wet bulb and dry bulb temperatures, both at the front of the oven and the back. After 11 minutes of baking, the cookies were immediately removed from the oven. The cookies were weighed following baking to determine moisture loss.

2.3 pH, water activity, and moisture content analysis

Cookie dough samples (raw and baked) were analyzed for pH, water activity, and moisture content. For pH measurements, a 5 gram sample of either raw or baked cookie dough was crumbled into 50 mL of deionized water and stomached (Stomacher® 3500, Seward) to combine at 150 rpm for 1 minute. A water activity meter (AquaLab Series 3, Decagon Devices, Pullman, WA) was used to determine the water activity of the cookie at 25 °C. A moisture meter (Millet Program, Moisture Analyzer HB43-S, Mettler Toledo) was used to determine the moisture content of the cookies. For analysis, each cookie was completely crumbled to obtain a random and even amount of crust and crumb sections of the cookie.

2.4 Microbial Analysis

Individual cookies (back, middle, and front) were transferred into separate Whirl-Pak filter bags containing 60 mL of 0.1% chilled peptone water and stomached at 150 rpm for 1 min (Stomacher® 3500, Seward). Raw dough samples (30 g) were prepared in an identical manner. The filtered portion was serially diluted in 0.1% peptone water and spread plated on Tryptic Soy Agar (TSA; Acumedia, Neogen Corporation) plates. Plates were incubated at 37°C for 24 hours prior to enumeration. Colonies with unique

morphologies were transferred to MacConkey Agar and Hektoen Enteric Agar (Acumedia) to characterize isolates. Colony morphology was evaluated following incubation at 37°C for 24 hours.

3. Results and Discussion

The raw company cookie dough had a pH of 6.56, a moisture content of 12.38%, and a water activity of 0.616 (Table 3.1). The microbial load of the raw cookie dough was 7.87 log CFU/g. One study analyzing survival of *Salmonella* in peanut butter cookies reported a similar pH value of 6.85 for the raw dough and slightly higher water activity value of 0.82 (Lathrop, et al., 2014). The low water activity of the dough prohibits the growth of bacteria; however, bacteria, including important foodborne pathogen such as *Salmonella*, can survive in low water activity foods for extended periods of time (Farakos et al., 2014).

The temperature profile of the baking treatment is shown in Figure 2.2. An expected steady progression of internal cookie temperatures as well as the difference between the wet bulb and dry bulb temperatures was evident during the baking treatment. In addition, the front cookie that was located closest to the oven door had the maximum temperature achieved from all three cookies. The maximum temperature of the three cookies baked at 163 °C ranged from 101 to 112 °C .

Baking led to a significant reduction of pH, moisture content, and water activity (Table 3.1). The moisture loss between the raw dough and the baked dough lessens the chance for microbial growth after heat treatment. Baking significantly reduced the microbial load

to 5.05 log CFU/g. These values demonstrate that baking the raw dough for 11 minutes at 163°C did not eliminate bacteria.

Table 2.1. Changes in water activity, pH, moisture content, and aerobic plate count of cookie dough and baked cookies treated at 163°C for 11 minutes.

	Raw Dough	**Baked Cookies**
Dough mass (grams)	30.00	28.79
Water Activity	0.616	0.587
pH	6.56	5.65
Moisture Content (%)	12.38	7.19
Aerobic Plate Count (Log CFU/gram)	7.87	5.05

Visual observation of the TSA plates clearly identified differences in the predominant bacterial populations of raw dough and baked cookies (Figure 2.2). The microbial load of raw dough was dominated by vegetative bacterial cells, whereas population of the baked cookies were primarily bacterial spores. This is most likely because the baking process killed the vegetative cells, but not the spore-forming cells.

The TSA plates from the raw dough had four distinct colony types (Figure 2.2; #1-4). The most abundant colonies in raw dough were cream colored, circular, and pinhead sized (#1), followed in number by colonies that were cream colored, circular, and pea sized (#2). The third most abundant (#3) were filamentous and grooved (indictative of sporeforming bacteria). These were the most abundance colony morphology associated with baked cookies. The fourth were the least abundant and were found on only three plates and were yellow, circular, and pinhead sized (#4). A significant difference between the colonies counted before and after baking was the morphology. One type of each of

the colonies was transferred to selective media to characterize as coliforms. Colony types #2 and #4 are can be classified as coliforms. Colony type #1 was capable of weak growth on these media, but unlikely to be a coliform. As expected, the sporeformer did not grow on either HE or EMB.

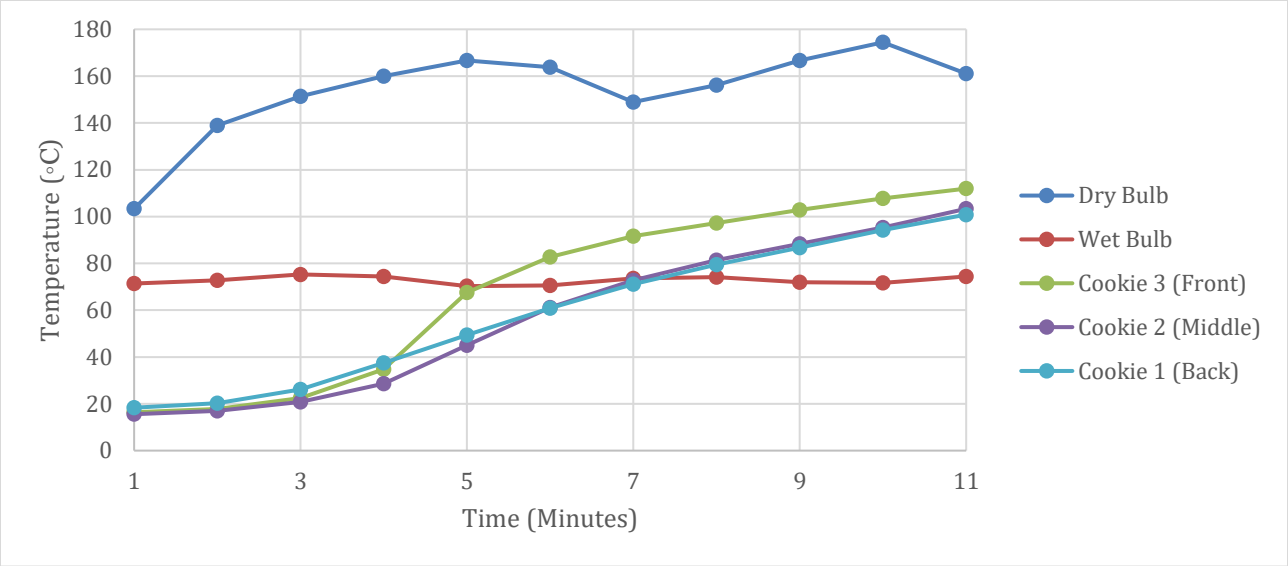

Figure 2.1. Temperature profiles of oven (dry and wet bulb) and internal cookies during baking process (325F, 11 min).

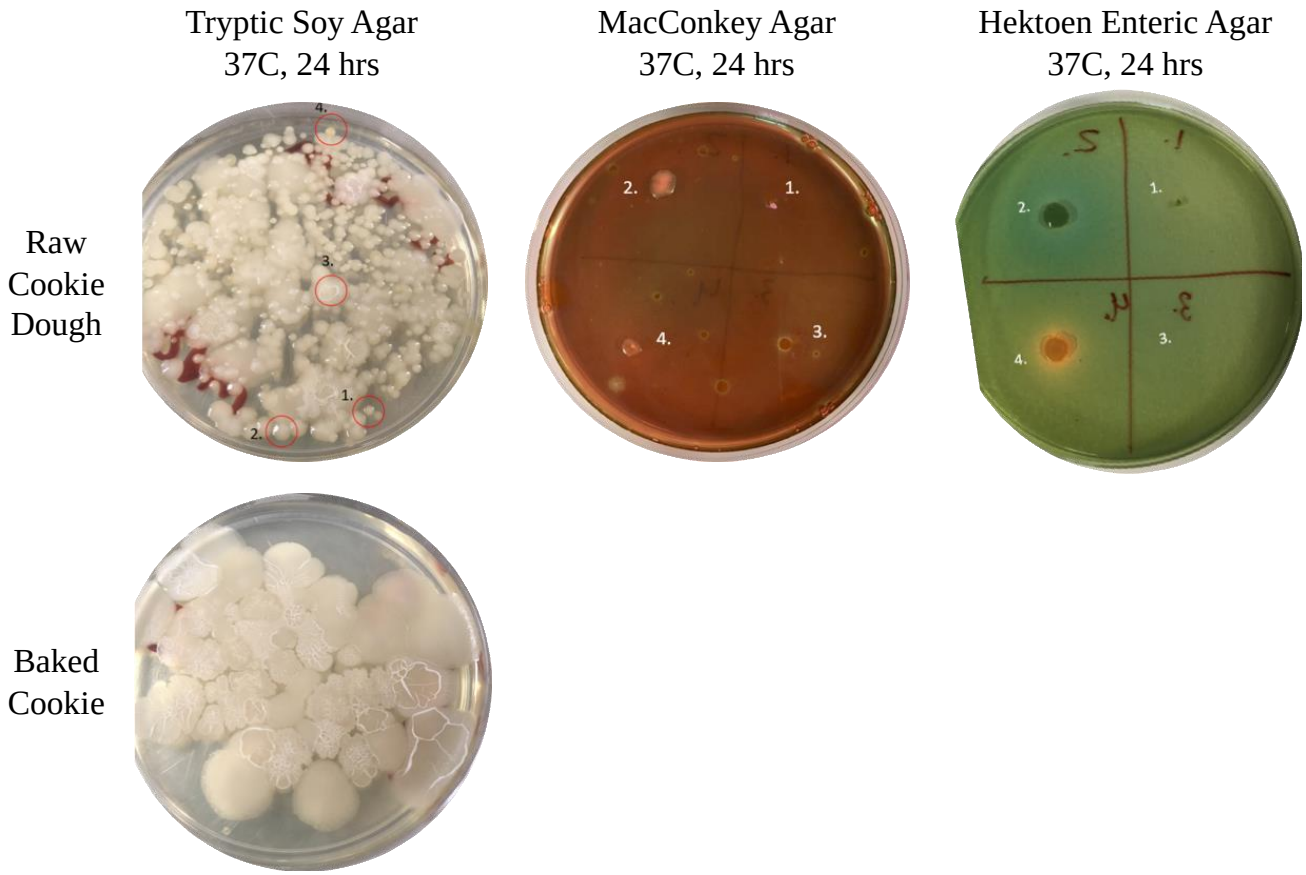

Figure 2.2. Colony morphologies associated with raw cookie dough and baked cookies.

4. Conclusion

Controlling coliform counts by baking is an important way that manufacturers maintain their quality standards. Validating the effectiveness of baking for controlling the coliform bacteria is a vital part of ensuring consumer safety. Knowing whether the contamination comes from before or after baking is an important part of identifying where the root cause of contamination is coming from. The results from this study showed that baking does reduce coliform counts, though it does not entirely eliminate them. Therefore, it can be concluded that the company supplying the cookie dough used in this study has an issue with post-cook cross contamination. Further investigation should be done in this company's processing plant to identify how to best control this contamination.

Acknowledgments

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Chapter 3. Validation of Baking as a Kill-Step for *Salmonella* and D-Value Determination for Inoculated Ingredients in Cookie Dough

Abstract

Several ingredients that are commonly used in baked products (i.e., flour, peanut butter, eggs, chocolate) have been linked to *Salmonella* outbreaks in recent years. Little information is available to demonstrate the efficacy of convection heating on baked products such as cookies. The efficacy of thermal treatments to reduce *Salmonella* is dependent on many variables, including the food matrix. The objective of this study was to determine the efficacy of baking (163°C, 11 min) to reduce *Salmonella* in cookie dough made with different contaminated ingredients. Flour, butter, eggs, peanut butter, and chocolate chips were inoculated with a *Salmonella* cocktail (serovars Newport, Typhimurium, and Senftenberg). The inoculated ingredients were used to prepare a basic sugar cookie recipe. *Salmonella* survivors were enumerated on both non-selective (Tryptic Soy Agar) and selective media (Hektoen Enteric Agar) for raw dough and baked cookies. In addition, D-values (60C) were calculated for doughs made with either inoculated flour, butter, eggs, and peanut butter. Baking effectively reduced *Salmonella* in cookies prepared with contaminated flour, butter, eggs, and peanut butter to reduce a microbial load in raw dough from a range of 7.857 to 7.952 log CFU/gram to 0 CFU/gram; however, identical baking conditions were less effective at reducing *Salmonella* in chocolate chips. In addition, D-values calculated for dough made with one of four inoculated ingredients (butter, flour, eggs, or peanut butter) showed that peanut

butter dough showed the most resistant to thermal treatments with a D-value of 43.3 minutes and flour showed least resistance to thermal treatments with a D-value of 35.5. Butter and eggs had D-values of 42.0 and 40.6, respectively. Performing baking validations and calculating D-values proved that thermal treatment at 163 degrees C was adequate for even the higher D-value ingredients, but was not adequate for control of *Salmonella* in chocolate chips.

1. Introduction

Salmonella, an enteric pathogenic bacterium, is a major cause of foodborne illness (Santos et al., 2001). The Center for Disease Control estimates that 1.2 million illnesses, 23,000 hospitalizations, and 450 deaths occur annually because of *Salmonella* in the United States. The Center for Disease Control specifies *Salmonella* serotypes Typhimurium, Heidelberg, Newport, Enteritidis, and Javiana as being the five most common serotypes present in foodborne illness outbreaks (CDC, 2015). It is commonly known that Salmonella can be contracted from raw eggs (Whiley and Ross, 2015, Outbreaks, Multistate Outbreak, The Associated Press, 2018), but it is less known that other ingredients used in baking can also harbor Salmonella. These ingredients include flour (Kenechukwu and Ndidi, 2015), dairy products (El-Gazzar and Marth, 1992), chocolate (D'aoust, 1977), and peanut butter (Cavallaro et al., 2011), amongst other ingredients such as spices, fruits, and coconut (Channaiah et al., 2016).

Over the years, *Salmonella* outbreaks have been linked to various bakery-style products including bread (Vo et al., 2014), flour-based raw baking mix (Eglezos, 2010) and cake-batter ice cream (Zhang et al., 2007). The outbreaks required hospitalizations, but no deaths occurred. Symptoms of *Salmonella* can range from temporary discomfort involving nausea and vomiting to more intense medical conditions, including severely dehydrating diarrhea (Mayo Clinic).

Salmonella control in processed foods in commonly accomplished using thermal treatments with temperature and time parameters that are predicted to provide an adequate reduction in contamination (commonly 5-log reduction). Often, the determination of effective time-temperature combinations are evaluated under ideal conditions (water bath) with little acknowledgment of other variables (humidity, air velocity, etc) that may influence thermal treatments. There is very little information in the literature to demonstrate the efficacy of convection baking to reduce *Salmonella* in low moisture products. With the implementation of the Food Safety Modernization Act (FSMA) in January of 2011 by President Obama, this research is especially important. The objective of FSMA is to improve the safety of the U.S. food supply by shifting the focus from a reactive approach to a preventive one. A key component of the Preventive Controls for Human Foods Rule involves the validation of process controls to demonstrate the efficacy of "kill-steps" to reduce pathogens (FDA Food Guidance Regulations). Scientific research for validation of these plans is important for industry to be able to prove that their preventive approach will accomplish and increase in the safety of their products. Previous research has demonstrated the efficacy of baking to reduce *Salmonella* in hamburger buns (Channaiah et al., 2016), muffins (Channaiah et al.,

2017), and dried eggs (Beloian and Schlosser, 1963). Few studies have analyzed how factors such as relative humidity affects the kill process of baking on *Salmonella*. One study done on inoculated hamburger buns demonstrated that relative humidity at levels greater than 3% are more affective at killing *Salmonella* (Shrestha et al., 2016).

In this study, we chose to investigate the efficacy of convection baking process on the inactivation of *Salmonella* in a basic sugar cookie recipe prepared with different contaminated ingredients (flour, egg, butter, peanut butter, or chocolate chips). We also sought to compare the thermal resistance of *Salmonella* in cookie dough made with either inoculated flour, egg, butter, peanut butter, or chocolate chips using standardized methods.

2. Materials and Methods

2.1 Design of Study and Analysis Method

This study contained five different ingredients used for inoculation: flour, butter, egg, peanut butter, and chocolate chips. Each inoculated product was incorporated into a separate batch of dough. Each batch of dough was baked and the inactivation of *Salmonella* was determined using standard spread-plating procedures. Trials using inoculated flour and butter were performed in duplicate. Trials using inoculated egg, peanut butter, and chocolate chips trials were performed once. Ingredient inoculation procedures, dough preparation, and sample analysis was performed in the Biosafety Level-2 food safety systems laboratory. All inoculated ingredients were handled within

the biological safety cabinet and adhered to the correct biosafety procedures, including smock, gloves, shoe covers, and eye protection. Data was analyzed using Excel.

2.2 *Salmonella* Cultures and Inoculum Preparation

Three *Salmonella* serovars, Senftenberg 775 W (ATCC 43845), Typhimurium (ATCC 14028) and Newport (ATCC 6962), were used in this project. These three serovars came from the culture collection in Dr. Joy Waite-Cusic's laboratory in the department of Food Science and Technology at Oregon State University. The cultures were cryogenically preserved at -80°C. Re-activation of cultures was achieved by culturing in 10 mL of Tryptic Soy Broth (TSB; Acumedia, Neogen Corporation) at 37 °C for 24 hours. Purity was verified by isolating on Hektoen Enteric Agar (HE; Acumedia) with incubation at 37°C for 24 hours. An individual colony from each strain was transferred cultured in TSB prior to spread plating (100 µl) on Tryptic Soy Agar (TSA; Acumedia, Neogen Corporation) create bacterial lawns. Harvesting the lawns was performed by adding 0.1% peptone water (1 ml; Acumedia) to the plate and gently scraping using sterile, disposable cell spreaders. This procedure was repeated twice per plate, using a total of 2 mL peptone water per plate. The culture solutions were pipetted into separate 50 mL conical tubes and stored at 4°C for up to one week. On the day of product inoculation, equal amounts of the three serovars were mixed together to create a *Salmonella* cocktail. Inoculum preparation was completed three different times to account for any variability associated with culture age and/or preparation (details in Appendix, Fig. 1).

2.3 Inoculations

2.3 a) Flour Inoculation

Enriched, unbleached all-purpose flour (Winco Foods, LLC, Boi) was used throughout this study. Flour (250 g) was weighed into a sanitized, sealable plastic tub (Rubbermaid, High Point, NC). A plastic atomizer spray head was used to spray approximately 2 mL (10 sprays) of the *Salmonella* cocktail into the flour. The lid was sealed and the flour was shaken to ensure even distribution of *Salmonella* inoculum and stored at 25-37°C for 1-3 days before use in the dough recipe.

2.3 b) Butter Inoculation

Salted butter (The Kroger Co., Cincinnati, Ohio) was used throughout this study. Butter (113 g) was removed from the refrigerator and tempered under ambient conditions for 2 hours. The butter was unwrapped and transferred into a sanitized glass jar. The *Salmonella* cocktail (1.8 mL, with or without 1% Tween 80) was mixed with the butter using either a pipette or a sterile spatula. The inoculated butter was stored at 25-37°C overnight prior to use in the dough formulation.

2.3 c) Egg Inoculation

Large, white grade AA eggs (The Kroger Co. Cincinnati, Ohio) were used for the dough. A single egg was cracked and a sterile plastic loop was used to break the yoke and mix the contents of the egg together in the shell. The egg was then transferred to a 50 mL conical. The *Salmonella* cocktail (1.8 mL) was added to the egg and vortexed for approximately 30 seconds. The inoculated egg was stored at 4-37°C for 1-3 days prior to use in the cookie dough.

2.3 d) Peanut Butter Inoculation

Creamy peanut butter (Jif, The J.M. Smucker Company, Orrville OH) was used in this study. Peanut butter (57 g) was transferred into a sanitized plastic jar. The *Salmonella* cocktail (1.8 mL) was stirred into the peanut butter using a sanitized spatula. The inoculated peanut butter was stored at 25 °C for approximately 16 hours prior to using in dough formulations.

2.3 e) Chocolate Chip Inoculation

Semi-sweet chocolate baking chips (The Kroger Co., Cincinnati, Ohio) were used in one batch of dough to create a chocolate chip cookie. Chocolate chips (200 g) were melted in a double boiler. The chocolate was allowed to cool for approximately 10 min prior to the addition of the *Salmonella* cocktail (1.8 ml). The mixture was stirred until well combined and transferred to a sanitized, plastic syringe to pipe into chocolate chips (100 g). The remaining chocolate was spread in a thin layer on parchment paper. Both the inoculated chips and chocolate sheet were transferred to the -80 °C freezer for 5 min to facilitate hardening. The chocolate sheet was fractured into chunks and the inoculated chips and chunks were incorporated into the dough formulation.

2.4 Cookie Dough Preparation

The cookie dough recipe was provided by Dr. Joy Waite-Cusic (Table 3.1). Inoculated dough was used for validation and D-value determination studies and non-inoculated dough was used for pH, water activity, and moisture content studies. In a biological safety cabinet, the butter and sugar were placed into a sanitized mixing bowl (Artisan®, KitchenAid®, St. Joseph, Michigan) and beaten using a sanitized paddle attachment at high speed for one minute. One egg was added and mixed on medium speed for 1 minute.

A sanitized spatula was used to scrape down the sides of the mixing bowl. Vanilla, salt and baking powder were added and the mixture was mixed on low to combine. Finally, flour was added as the last step and mixed on low until combined. The spatula was used to scrape the bowl as needed. The dough was weighed into cookie dough balls of 30 ± 1 gram and placed on a sheet of parchment paper (Reynolds Cookie Baking Sheets) on top of a sanitized metal cookie sheet. The cookies were flattened slightly, to approximately 5.0 centimeters in diameter and 2.0 centimeters tall. Twelve cookies were placed onto each sheet.

Table 3.1. Cookie formulation used in this study.

Ingredient	**Weight (grams)**
Butter	113
Peanut Butter[a]	56
Granulated Sugar	100
Large Egg	55
Vanilla	5
Baking Powder	5
Salt	2
Enriched Flour	225
Chocolate chips/chunks[b]	200

[a]Peanut butter was only used in select dough formulations. When peanut butter was used, salted butter content was reduced by 50%.

[b]Chocolate chips were only used in select dough formulations. Dough formulation remained the same regardless of use of chocolate chips.

2.5 Cookie Baking and Temperature Measurement

A counter sized convection oven (Waring Commercial Half-Size Heavy-Duty Convection Oven, WCO500X, 1.5 cubic feet, Torrington, CT) was preheated to 163 °C for at least 30 minutes prior to baking. Data logger thermocouples (Easylog EL-USB-TC, Lascar Electronics, Erie, PA) were used throughout the oven and in the cookies to monitor and record temperatures. Four thermocouples were used in the oven to monitor

dry bulb and wet bulb temperatures in the oven chamber. Three thermocouples were used to record internal temperature of the cookies. For all validation trials, the thermocouples were located in the geometric centers of the three cookies on the middle right side of the oven as shown in Figure 3.1. Thermocouple 1 was inserted into the cookie at the back of the oven, thermocouple 2 into the cookie in the middle of the oven, and thermocouple 3 in the cookie at the front of the oven, closest to the door. The thermocouples monitored both the oven and cookie temperatures by taking temperature measurements every 10 seconds. The oven was then shut and left undisturbed for 11 minutes. After 11 minutes, the cookies were removed from the oven.

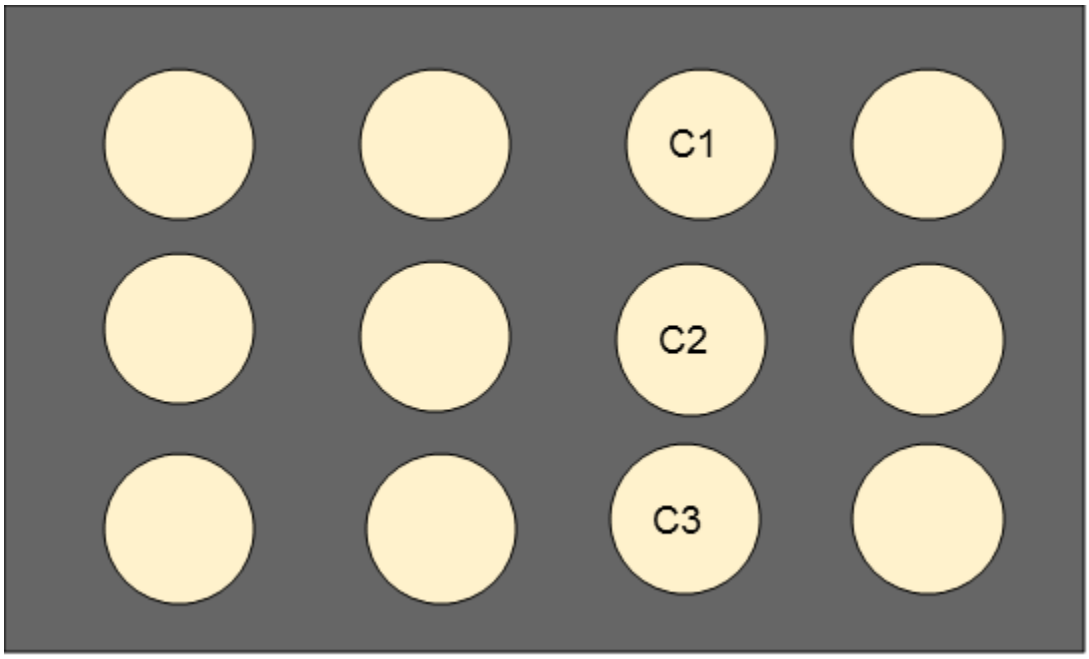

Figure 3.1: Cookie placement and thermocouple positions from back of oven (C1) to front of oven (C3).

2.6 pH, water activity, and moisture content analysis

For the pH, water activity, and moisture content analysis, uninoculated dough was used to take measurements. Measurements were taken on raw cookie dough and fully baked cookies. The tests used the cookie in the middle of the middle right hand column, C2, as shown in Figure 1. The pH meter was calibrated using 4.0 and 7.0 standard buffer solutions. The total cookie pH was measured at approximately 25°C. Raw or baked cookie dough (5 g) was crumbled into 50 mL of deionized water. A water activity meter (AquaLab Series 3, Decagon Devices, Pullman, WA) was used to determine the water activity of the cookie at 25°C. A moisture meter (Moisture Analyzer HB43-S, Mettler Toledo) was used to determine the moisture content of the cookies. For analysis, each cookie was completely crumbled in order to obtain a random and even amount of both the crust and the crumb of the cookie.

2.7 Microbial Analyses

After baking, the cookies were immediately submerged into 60 mL of chilled 0.1% peptone water in a WhirlPak filter bag (Whirl-Pak® Filter Bags for Homogenizer Blenders, Sterile, Nasco®, Modesto, CA) to prevent further baking. The cookie and peptone water mixture was then stomached (Stomacher® 3500, Seward) at 150 rpm for one minute in order to thoroughly homogenize. In addition, 30 grams of raw dough were stomached with 60 mL of 0.1% peptone water to obtain pre-bake plate counts for the dough.

2.8 D-value determination

In order to determine D-values, thermal inactivation curves for *Salmonella* were analyzed. Cookie dough was prepared in four separate batches using inoculated flour,

eggs, butter, and peanut butter using formulations described in Table 3.1. Dough (5 g) was transferred into a WhirlPak filter bag and flattened to create a thin layer to make the heat-up time of the dough negligible. The bags were placed into a water bath (ANOVA, Chemyx, Stafford, TX) that was preheated to 60°C. Dough was treated at 60°C for 1, 20, 40, 60, and 80 min. At each time point, three samples were removed and plunged into an ice bath to prevent further cooking. Diluent (10 ml of 0.1% peptone water) was added to each WhirlPak bag and the contents were stomached as previously described. Raw dough was handled in a similar manner.

2.9 Microbial Analysis

Raw cookie dough or baked cookies were homogenized with 0.1% peptone water (1:2) by stomaching at 150-180 rpm for 1 min. Serial dilutions were created in 0.1% peptone water and spread plated onto selective (Hektoen Enteric Agar (HE)) and non-selective media (Trypticase Soy Agar (TSA)). All plates were incubated at 37 °C for 24 hours prior to enumeration. Microbial data from water bath treatments were used to calculate D-values at 60C. Linear regression graphs were made in Microsoft Excel 2016 and D-values were calculated using the absolute values of the inverse of the slopes of the regression lines of log CFU/g of the *Salmonella* cocktail.

3. Results and Discussion

The raw cookie dough was characterized by a pH of 6.70, aw of 0.817, and moisture content of 17.565% (Table 3.2). The relatively low water activity (<0.92) of the cookie dough would prevent *Salmonella* growth in the absence of temperature control; however, *Salmonella* is quite capable of surviving in this type of environment for long periods of

time. This makes ensuring kill of the *Salmonella* through correct time and temperature parameters vital to ensure food safety. In addition, the heat resistance of *Salmonella* increases as the water activity of the food decreases (Salmonella (non-typhoidal), 2013).

A representative thermal profile of the baking process is shown Figure 3.2. The wet bulb temperature remained steady throughout the 11-min cooking period, whereas the dry bulb temperature dipped when the oven door was opened and did not restore to the targeted 163°C temperature until around 4 minutes into baking. The relative humidity in the oven was calculated to be ~4%. There was limited variability in final product temperature due to spatial arrangement in the oven; however, cookies placed at the front of the oven (nearest to the door) experienced the quickest increase in temperature.

Baking the cookies for 325 °F for 11 minutes produced an optimum result in terms of color and texture for the cookies. The baked cookies were slightly browned on the bottom with only hints of browning on top and fully cooked in the center. These time and temperature parameters produced a cookie that resembled a product typical of industry standards. Typical baking temperatures for commercial cookies production range from 160 to 180°C with baking times between 7 and 16 min (Manley, 1998). The time and temperature values used in this study fit within these ranges.

The baked cookie was elevated in pH to 7.44 with a reduced water activity of 0.679 and a moisture content of 14.503% (Table 3.2). With an already low moisture content, baking

the cookies resulted in a 3.062% loss in moisture (Table 3.2), resulting in an even lower moisture food that is less suitable for *Salmonella* growth.

Table 3.2. pH, moisture content, and water activity of raw cookie dough and baked cookies (results from dough made with butter instead of peanut butter and no chocolate chips added, 163C, 11 min).

	Raw Dough	**Baked Cookie**
pH	6.70	7.44
Moisture Content (%)	17.565	14.503
Water Activity	0.817	0.679

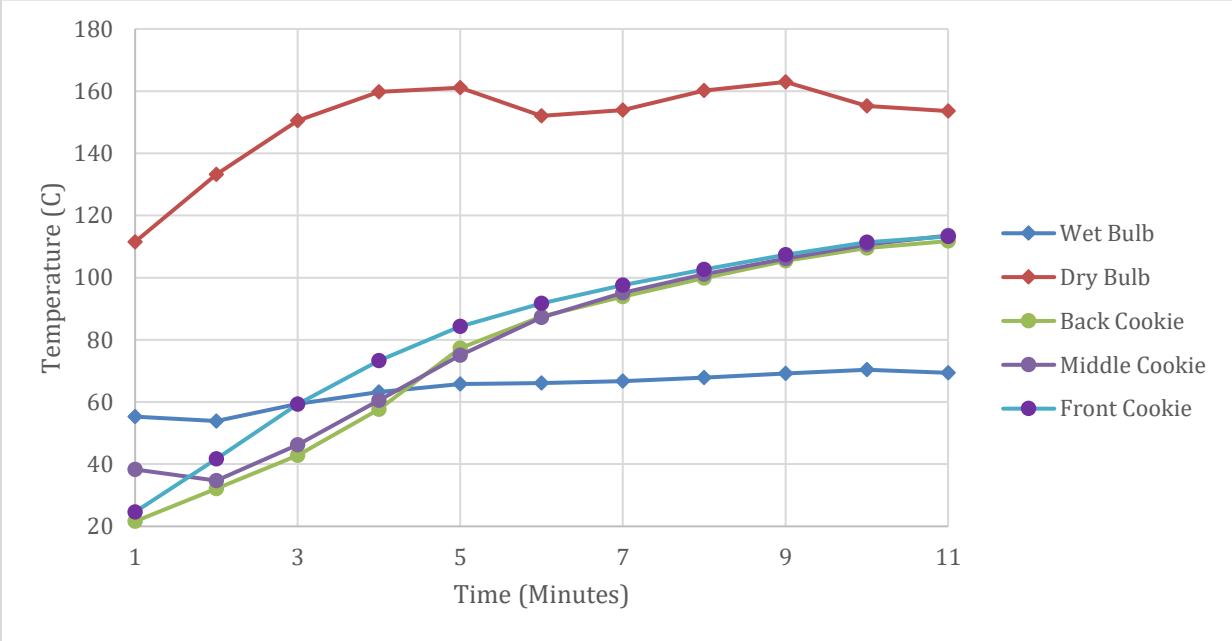

Figure 3.2: Mean baking temperature profiles for the oven chamber (dry bulb and wet bulb) and internal cookie temperature at the back, middle, and front of the oven baked at 163°C for 11 min.

The *Salmonella* cocktails ranged between 11.7 and 12.9 log CFU/ml. Contamination levels in inoculated raw cookie dough ranged from 6.37 log CFU/g (chocolate chips) to 7.95 log CFU/g (flour). *Salmonella* levels in baked cookies varied substantially depending on the ingredient that was inoculated (Figure 3.3). *Salmonella* survivors were below the detection limit (<3 log CFU/g) for dough prepared with contaminated flour, egg, and peanut butter. Very low levels of survivors (<0.8 log CFU/g) were found after baking cookies made with contaminated butter. Interestingly, the baking process was only minimally effective at reducing *Salmonella* populations in cookies made with contaminated chocolate chips/chunks. Before baking, the population level in the raw dough made with inoculated chocolate chips was 6.37 log CFU/gram and finished cookies contained *Salmonella* survivors at 5.94 log CFU/gram.

A study on *Salmonella* inoculated hamburger buns with inoculated sesame seeds found that maintaining relative humidity at 20% during baking produced greater lethality on *Salmonella* than relative humidity that was ≤ 3% (Shrestha et al., 2016). Reassuringly, the study also found that even with a less lethal treatment, a lower relative humidity still produced ample kill of *Salmonella* in the treated populations (≥ 5 log decrease). The relative humidity calculated using average wet and dry bulb temperatures from 3-6 different trials at a target oven temperature of 325 °F with approximately 360 grams of cookie dough with an Aw value of 0.817 and a moisture content of 17.565 was found to be 4% (Calculation 1). This relative humidity percentage is close to the ≤ 3% value that was found to be less lethal to *Salmonella*, but the results from the study give proof that even with a lower relative humidity, the end result of the kill-step was achieved. In this study, it was shown that with a relative humidity of 4% proper lethality was achieved in

flour, egg, butter, and peanut butter trials but not chocolate. Further research would need to be done in this area to test for the effect of relative humidity on *Salmonella* in chocolate.

The inoculated chocolate chip trial exhibited the least amount of reduction in *Salmonella*. There are several factors of the study that could aid in explaining the viability of *Salmonella* in chocolate chips. First, the log CFU/gram for the raw dough are not accurate. Because the dough was only stomached with the peptone water, the chocolate chips were largely left unscathed and were not fully incorporated into the mixture. The original mixture for the raw dough resembled milk; it was white and opaque, but no pieces of chocolate made it through the WhirlPak filter bags. This resulted in recorded CFU/gram that was most likely much lower than it should have been. After baking, when the chips had melted, they were easily incorporated into a mixture with the peptone water. The resulting mixture appeared like chocolate milk and gave a clear image of the amount of chocolate that was in the filtered mixture. These results gave a more accurate image of how much *Salmonella* was truly in the dough.

However, while this answers the question of why the initial CFU/gram count was lower in the raw dough than it should have been, it does not answer the question of how the *Salmonella* cocktail was able to survive in such greater quantities in the chocolate chips as compared to the flour, butter, eggs, and peanut butter. One reason for this could have been the structure of the chocolate chip acting as a thermal barrier between the cookie and the *Salmonella* in the chip. Perhaps the chocolate chips were slower to heat than the rest of the cookie dough and were not able to reach the internal temperatures

that the cookies reached. Though no water was added to the dough, the eggs and butter both contribute enough water to the dough to raise the water activity level to 0.817. Chocolate, on the other hand, is an extremely low water activity ingredient, and has a measured Aw value of 0.4-0.5 (Novasina, 2013). Although having a low water activity may sound desirable for decreasing the water availability for *Salmonella* to thrive in, it might cause the ingredient matrix to heat more slowly than those that contained more water. In addition, it has been found that the heat resistance of Salmonella increases as the water activity of the food decreases and that high fat, low-moisture foods, including chocolate and peanut butter, can give protection to *Salmonella* against heat (Salmonella (non-typhoidal), 2013). The combination of a low water activity value and a high fat percentage of the ingredient has been shown to raise the tolerance of Salmonella towards thermal treatments. Temperatures that are able to kill *Salmonella* in other ingredients may not work as effectively on chocolate. This could explain the survival of the *Salmonella* inoculum in chocolate chips as compared to the rest of the ingredients (Finn et al., 2013). Survival of *Salmonella* in low-moisture foods can be contributed to factors including filamentation of cells, osmoprotectant metabolites, and ability to convert to metabolically dormant states (Finn et al., 2013).

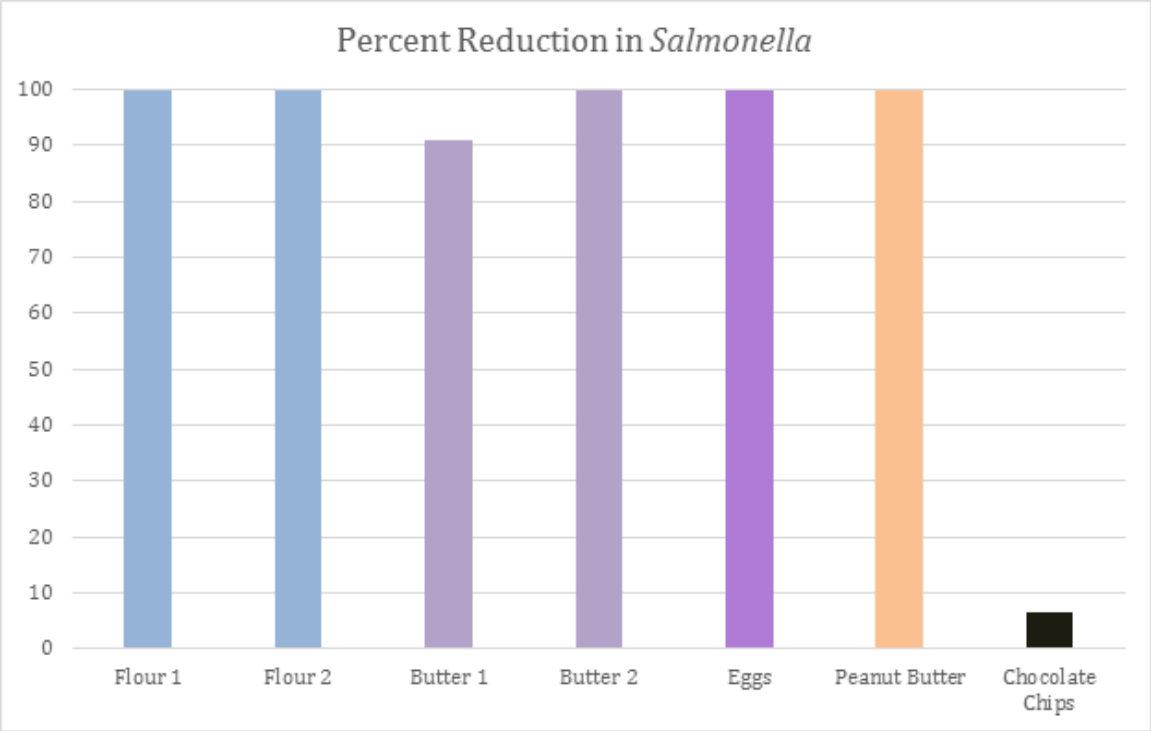

Figure 3.3: Percent reduction in *Salmonella* in seven batches of dough, each using a different inoculated ingredient. Results obtained from enumeration of raw dough and fully baked (325 °F for 11 minutes) cookies.

Thermal inactivation curves that were used to determine D-values for cookie dough using each individual inoculated ingredient (Figure 3.4). The resulting D-values for the doughs using the inoculated ingredients can be seen in Table 3.5. The ingredient with the lowest D- value of 35.46 minutes was flour. The middle D-values came from eggs and butter, coming in at 40.65 and 42.02 minutes respectively. The largest D-value resulted from peanut butter and was 43.29 minutes.

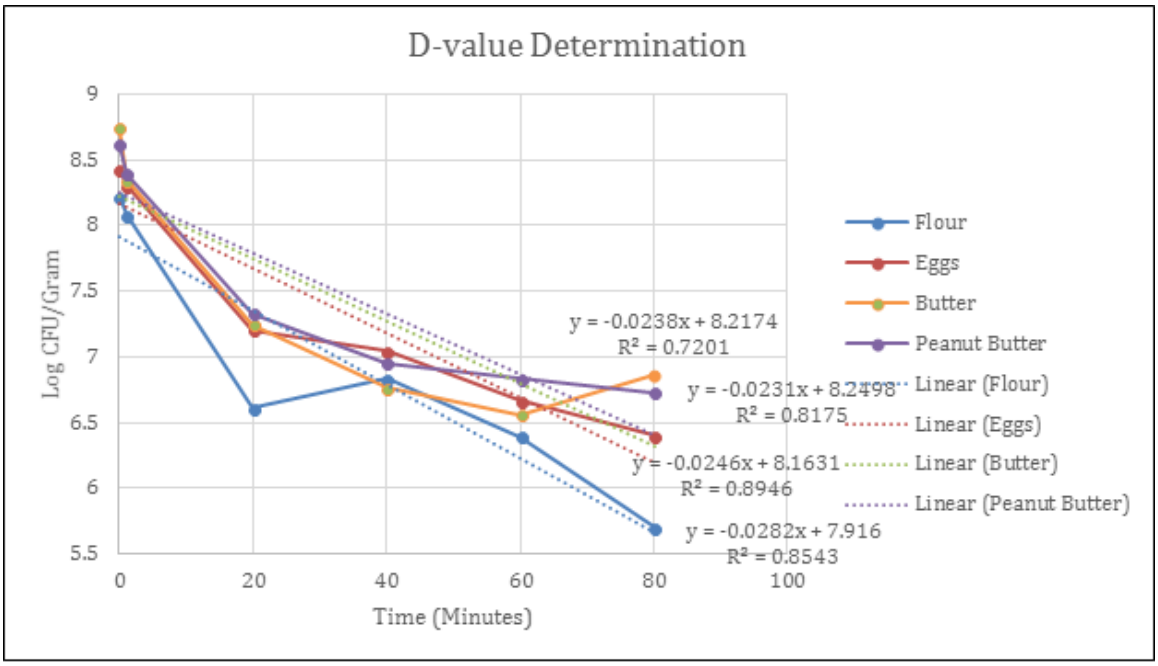

Figure 3.4. Thermal inactivation curves in a 60°C water bath of a 3-strain *Salmonella* cocktail in four batches of cookie dough, each containing a different inoculated ingredient. Results enumerated from raw dough and dough sampled at 5 progressing time points on non-selective media (Tryptic Soy Agar).

Table 5: $D_{60°C}$ -values for a 3-strain *Salmonella* cocktail in cookie dough made from different contaminated ingredients.

Inoculated Ingredient	**$D_{60°C}$ -value (min)**
Flour	35.5
Egg	40.6
Butter	42.0
Peanut Butter	43.3

5. Conclusions

Control of *Salmonella* in baked products is vital for the safety of consumers. Validation processes are essential for maintaining effective food safety plans that contain kill-step procedures. Baking cookies at 325°F for 11 minutes acted as an effective kill-step when

the dough was inoculated with flour, butter, eggs, and peanut butter. These time and temperature parameters resulted in a minimum of a 7-log reduction in a 3-serovar *Salmonella* cocktail. Further studies would need to be performed on chocolate chips to determine what validation parameters would kill *Salmonella* in those specific ingredient matrices. Additionally, the D-values obtained from this study indicate that at 60°C, peanut butter shows the most resistance to heat treatments of *Salmonella*¸ followed by butter, eggs, and flour. Further studies should be performed to determine D-values at various temperatures and on ingredients such as chocolate chips.

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4. Conclusion

The importance of controlling bacteria in bakery products cannot be overstated. Because of their massive presence in the American diet and the nature of the contamination of their ingredients, emphasis on food safety must be a priority of the bakery industry. One of the most important food safety aspects to focus on is the heat treatment used in bakery products as the lethal treatment for any type of bacterial contamination, including coliform and *Salmonella* contamination. In company supplied cookie dough with high coliform counts, it was found that baking 30-gram portions of dough at 163 ℃ mostly, though not entirely, eliminated coliforms and that further investigation must be done in the processing plant to identify the source of post-bake contamination. In cookies inoculated with known concentrations of a three-serovar *Salmonella* cocktail inoculum, baking 30-gram portions at 163 ℃ for 11 minutes validated control of *Salmonella* in dough that had been made with inoculated flour, eggs, butter, and peanut butter. This was proven by a minimum 7-log CFU/gram reduction from the raw dough to the baked cookie samples. In addition, D-values showed that even the inoculated peanut butter dough that showed the most resistance to heat treatment with a D-value of 43.3 minutes was controlled by the 163 ℃ for 11 minutes baking parameters. However, these same parameters were not able to control inoculated chocolate chips due to their difference in matrix make-up and structure. Further research should be done on chocolate chips to determine the most effective way to control *Salmonella* in chocolate. Additionally, further research should be conducted to determine validation for a wide variety of baked goods to ensure consumer safety in all bakery products.

Appendix.

The dough used for validation of baking and D-value determination differed between the flour trials 1 and 2. A third batch of inoculated flour was used for D-value determination for flour. Similarly, the batches between batch 1 of butter (without tween, used for validation) and batch 2 (with tween, used for validation and D-value determination) were two different batches. For eggs, batch 1 (validation) was different from batch 2 (D-value). For peanut butter, the same dough was used for both the validation and the D-value determination. For chocolate chips, only validation was tested and no D-value determinations were done.

Salmonella Cocktail mixtures used for trials for both Validation and D-value determination									
Salmonella Cocktail Number	Flour 1 (Validation)	Flour 2 (Validation)	Flour 3 (D-value)	Butter 1 (Validation)	Butter 2 (Validation and D-value)	Egg 1 (Validation)	Egg 2 (D-value)	Peanut Butter (Validation and D-Value)	Chocolate Chip (Validation)
Salmonella **Cocktail 1: Lawns Harvested 3/5/2018**	X					X			
Salmonella **Cocktail 2: Lawns Harvested 3/22/2018**		X		X					
Salmonella **Cocktail 3:**			X		X		X	X	X

Lawns Harvested 4/25/2018									

2.3 a) Flour Inoculation

Enriched, unbleached all-purpose flour (Winco Foods, LLC, Boi) was used throughout this study. Flour (250 g) was weighed into a sanitized, sealable plastic tub (Rubbermaid, High Point, NC). An atomizer was used to spray approximately 2 mL (10 sprays) of the *Salmonella* cocktail into the flour. The lid was sealed and the flour was shaken to ensure even distribution of *Salmonella* inoculum and stored at 25-37°C for 1-3 days before use in the dough recipe.

2.3 b) Butter Inoculation

Salted butter (The Kroger Co., Cincinnati, Ohio) was used throughout this study. Butter (113 g) was removed from the refrigerator and tempered under ambient conditions for 2 hours. The butter was unwrapped and transferred into a sanitized glass jar. The *Salmonella* cocktail (1.8 mL, with or without 1% Tween 80) was mixed with the butter using either a pipette or a sterile spatula. The inoculated butter was stored at 25-37°C overnight prior to use in the dough formulation.

2.3 c) Egg Inoculation

Large, white grade AA eggs (The Kroger Co. Cincinnati, Ohio) were used for the dough. A single egg was cracked and a sterile plastic loop was used to break the yoke and mix the contents of the egg together in the shell. The egg was then transferred to a 50 mL conical. The *Salmonella* cocktail (1.8 mL) was added to the egg and vortexed for

approximately 30 seconds. The inoculated egg was stored at 4-37°C for 1-3 days prior to use in the cookie dough.

2.3 d) Peanut Butter Inoculation

Creamy peanut butter (Jif, The J.M. Smucker Company, Orrville OH) was used in this study. Peanut butter (57 g) was transferred into a sanitized plastic jar. The *Salmonella* cocktail (1.8 mL) was stirred into the peanut butter using a sanitized spatula. The inoculated peanut butter was stored at 25 °C for approximately 16 hours prior to using in dough formulations.

2.3 e) Chocolate Chip Inoculation

Semi-sweet chocolate baking chips (The Kroger Co., Cincinnati, Ohio) were used in one batch of dough to create a chocolate chip cookie. Chocolate chips (200 g) were melted in a double boiler. The chocolate was allowed to cool for approximately 10 min prior to the addition of the *Salmonella* cocktail (1.8 ml). The mixture was stirred until well combined and transferred to a sanitized, plastic syringe to pipe into chocolate chips (100 g). The remaining chocolate was spread in a thin layer on parchment paper. Both the inoculated chips and chocolate sheet were transferred to the -80 °C freezer for 5 min to facilitate hardening. The chocolate sheet was fractured into chunks and the inoculated chips and chunks were incorporated into the dough formulation.

Results for the average weight lost per cookie during baking were obtained by recording weights of raw cookies and baked cookies. Averages were taken from three cookies from batches containing one of four inoculated ingredients: flour, butter, egg, or peanut butter. Table 6 shows that the average weight lost during baking for all cookies was 1.587

grams. Inoculated flour, butter, and peanut butter averaged weight losses of 1.635, 1.687, and 1.587 grams respectively, whereas inoculated eggs only experienced an average weight loss of 1.289 grams (Table 4).

Using the calculations presented below in Calculation 1, the relative humidity, using averages of wet bulb temperatures taken from three separate baking trials and dry bulb temperatures taken from six separate baking trials, was calculated to be 4%.

$$147.723\,°\mathrm{F} = 64.2929\,°\mathrm{C}$$
$$302.667°\mathrm{F} = 150.370°\mathrm{C}$$
$$Assuming\ pressure = 101.3\ kPa$$
$$A = conversion\ factor = 0.00066(1.0 + 0.00115T_{wb})$$
$$e_d = water\ vapor\ pressure$$
$$es_{wb} = saturation\ vapor\ pressure$$
$$T_{db}: es_{db} = e^{\left[\frac{16.78\,T_{db} - 116.9}{T_{db} + 237.3}\right]}$$
$$e_d = es_{wb} - AP(T_{db} - T_{wb})$$
$$RH = 100\frac{e_d}{es_{db}} = 4\%$$

Calculation 1

AVERAGE WEIGHT LOST DURING BAKING (GRAMS)	
FLOUR	1.635
EGGS	1.289

BUTTER	1.687
PEANUT BUTTER	1.587
AVERAGE FROM ALL TRIALS	1.587

`

Table 4: Weights lost during baking at 325 °F for 11 minutes averaged from three cookies per trial of inoculated ingredient.