VALIDATION OF A SIMULATED COMMERCIAL PLAIN BAGEL BAKING PROCESS TO CONTROL *SALMONELLA* AND SHIGA TOXIN PRODUCING *ESCHERICHIA COLI*

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by

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The undersigned, appointed by the dean of the Graduate School, have examined the thesis entitled

VALIDATION OF A SIMULATED COMMERCIAL PLAIN BAGELS BAKING PROCESS TO CONTROL *SALMONELLA* AND PATHOGENIC SHIGA TOXIN PRODUCING *ESCHERICHIA COLI*

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ABSTRACT

Flour is a minimally processed raw agricultural product with low water activity and is typically not conducive to bacterial growth. Despite this, pathogenic microorganisms such as *Salmonella* and STEC can withstand the drying process and persist in flour for weeks and even months in a desiccated state. Both *Salmonella* and STEC have been associated with several foodborne illness outbreaks linked to flour and its derivatives in the United States. The FDA Food Safety Modernization Act (FSMA) mandates validation of preventive controls, such as baking, to control potential foodborne pathogens and assure the safety of finished food products. To the best of our knowledge, there have been no prior baking studies on thermal inactivation of *Salmonella* and STEC in plain bagels. Therefore, studies were conducted to validate the effectiveness of plain bagels baking process ((450°F) for 14 minutes) as an effective kill-step for controlling *Salmonella* and STEC in the event of pre-baking contamination. Additionally, water activity and pH in plain bagels during baking, and thermal inactivation kinetics (*D*- and *z*-values) of *Salmonella* and STEC in plain bagels dough were studied. The results clearly demonstrated that baking plain bagels at 450°F for at least 14 minutes will result in a >5 log reduction in *Salmonella* and STEC population thus providing an effective kill-step assuring the safety of the finished food products. The *D*- values of *Salmonella* in plain bagels at, 56, 59, and 62°C were 24.5 ±1.5, 13.3 ±1.85, and 6.8 ± 1.05 min with a *z*- value of 11.1 ±1.58° C. The *D*- values of STEC in plain bagels at, 56, 59, and 62°C were 26.3 ±1.55, 9.0 ±0.27, and 2.50 ±0.15 min with a *z*- value of 5.8 ±0.16°C.

CHAPTER 1

REVIEW OF LITERATURE

Food Safety Regulations in the United States

Back in 1862 President Lincoln established the USDA, Unites States Department of Agriculture (USDA, 2018). This was only the beginning of the trend towards safer and better regulated food. In 1906 under the Federal Food and Drugs Act the then Bureau of Chemistry officially became what we know as the Food and Drug Administration (FDA) (Swann, 2017). In America, these two agencies are the ones who oversee the protection of our nation's food supply. Throughout their history there has been back and forth on what fell under their authority and how much sway they had on private industry. This power struggle changed in 2011 with the establishment of the Food Safety Modernization Act (FSMA) (FDA, 2023a).

On January 4th, 2011, President Obama signed in this law that handed additional regulatory power to the FDA. The act enables the FDA to focus more on preventing food safety issues rather than relying primarily on reacting to them after they occur. Following the FSMA act, the FDA has gained the power to hold companies accountable for food safety failures. Additionally, the FDA gained the authority to require companies to recall hazardous food products from commerce, gained more leeway in their inspections and requirements, and finally companies were required to comply with the regulations the FDA set for prevention-based controls. According to the FDA, food processors must validate preventative control steps for food safety and their associated preventive

measures. In order to comply with FSMA it is required that these companies provide adequate scientific documentary evidence that their kill steps are sufficient. These controls/validations are another way the FDA is working to improve food safety in United States thus protecting consumers from foodborne illness outbreaks.

Although the introduction of FSMA has led to an increase in food safety parameters, foodborne illness outbreaks still persist across the United States. An estimated 600 million people fall ill due to the consumption of contaminated food each year with an estimated 420,000 of those individuals dying every year due to contaminated food (WHO, 2022). The truth is that all of these outbreaks are largely preventable. The primary cause of all these outbreaks is improper food handling and/or improper food processing (Partnership for Food Safety and Education, 2018).

Food manufacturing facilities that must register under section 415 of the Food, Drug, and Cosmetic Act must follow the requirements of risk-based preventive controls, as mandated by FSMA to prevent accidental or intentional adulteration of finished food products (FDA, 2023b). The preventive control requirements in 21 CFR part 117 must be validated by facilities subject to the FSMA act to control the hazard as appropriate to the nature of the preventive control and its role in the facility's food safety system (FDA, 2023b).

The U.S. baking industry has a relatively safe record for producing shelf-stable processed bakery food products. However, the shelf life of bakery products has often been hampered by microbiological spoilage, leading to significant economic losses for bakeries. Although not directly linked to production, there were 150 outbreaks and 3,506 illnesses related to baked goods reported in the U.S. between 2001 and 2010 (CSPI,

2013). In April of 2023 there was a multistate *Salmonella* outbreak of Gold Medal bleached and unbleached flour (FDA, 2023c). It resulted in 14 illnesses and 3 hospitalizations from various baked goods. Pathogens such as *Salmonella* spp., Shiga-toxin producing *Escherichia coli* (STEC) and other potential foodborne pathogens can be introduced into bakery products through a wide range of ingredients such as milk products, flour, chocolate, egg, nuts, coconut, peanut butter, fruits, vegetables, spices, yeast flavorings, streusel toppings, human error and other environmental factors (USDA, 2012). Although *Salmonella* spp. and STEC cannot grow in foods or ingredients with low-water activity, they can survive for several months, even years, in low-water activity foods and in dry environments (Beuchat et al., 2013). Growth could occur when favorable conditions prevail, such as rehydration of flour during batter or dough preparation. Over 2000 bakery products are available on the market, each with a different level of sugar, fat, and other ingredients. The presence of pathogens such as *Salmonella* spp., and STEC in the finished baked product could create a public health risk if thermal processing steps or preventive controls such as baking or cooking are not adequate. Therefore, it is critical for bakers to implement validated baking preventive control(s) to ensure the safety of the finished food products.

Foodborne Illness

Foodborne illness has always had an impact on peoples' lives. Foodborne illness is caused by the consumption of contaminated food, water, contact with animals/their environment, or even from contact with other people (Minnesota Department of Health,

2022). There are also multiple causes of foodborne illnesses, it could be bacterial, viral, or come from a parasite. The one thing that they all have in common is that the food/water source was not properly treated to fully dispose of the causative agent. Some common symptoms of foodborne illnesses include diarrhea, vomiting, nausea, fever, abdominal cramps, joint/back aches, and fatigue (Minnesota Department of Health, 2022).

Foodborne illnesses can have very different symptoms and severity depending on the causative factor, dose, and the person infected. Symptoms can vary all the way from mild discomfort to life threatening. Specifically, those with weakened immune systems as well as children and the elderly are at the greatest risk of the worst-case scenarios with foodborne illnesses (FDA, 2022). The FDA lists many types of foodborne illness causing organisms such as *Salmonella*, *E. coli* O157:H7, *Listeria monocytogenes*, *Staphylococcus aureus*, and more. To ensure the safety of a finished product, it is important to consider the different ways bacteria can affect the consumers from different sources.

Salmonella

As one of the leading causes of illness and the leading cause of hospitalizations and deaths, salmonellosis is a major public health menace. Salmonellosis is a bacterial disease caused by *Salmonella* pathogen affecting the intestinal tract with the main source of infection being the ingestion of contaminated food or water (Mayo Clinic, 2022). Symptoms vary by individual, some people have no symptoms, while some may have diarrhea, fever, nausea, vomiting, chills, stomach cramps, headache, or blood in their

stool. Symptoms can start anywhere from 6 hours post consumption of contaminated food to 6 days and last anywhere from ~8 hours to a week (Mayo Clinic, 2022). In most individuals' treatment or hospitalization isn't necessary, but it can be necessary when the infection lasts multiple days, dehydration is severe, in the case of bloody stools or a high fever. *Salmonella* is capable of infecting people through food in multiple ways. Some common ones include raw meat (like poultry or seafood), raw/undercooked eggs, unpasteurized dairy products, fruits and vegetables, improper food handling, infected surfaces and via infected animals (Mayo Clinic, 2022).

There is another vector through which *Salmonella* can infect people through, that is flour. Flour has been the culprit for multiple foodborne outbreaks in the US (Moyer, 2023). Moyer also mentions that the grain can be contaminated in the field as it grows, either from contaminated water or via fecal matter containing *Salmonella*. Additionally, Moyer mentions that the grain tempering and milling steps involved in further processing flour are not bactericidal, and neither step has an impact on the possible bacterial presence. Meaning that an adequate heat-kill step is required to prevent illness by downstream food processors, such as bakeries.

Shiga Toxin Producing *Escherichia coli*

Another common and possibly deadly cause of foodborne illness is pathogenic *Escherichia coli,* specifically Shiga Toxin Producing *Escherichia* coli (STEC). With the sources of *E. coli* being the intestines of animals, contamination of foods and water occurs when fecal matter containing pathogenic *E. coli* comes into contact with them

(California Department of Health, 2023). The toxin produced by STEC has the potential to make individuals extremely sick. Some of the symptoms include vomiting, abdominal cramps, and bloody or non-bloody diarrhea. Severe cases can lead to hemolytic uremic syndrome (HUS) which can result in damage of red blood cells, kidney failure, and possibly death. Estimations as high as 1 in 10 individuals infected with STEC bacteria can develop HUS. The possible sources of contamination are raw/undercooked beef products, raw produce, unpasteurized milk and juices, contaminated water, as well as raw flour.

A study by Crowe et al. (2017) found that a total of 56 cases of STEC infections occurred in the US in 2016, which represents 25% of the individuals who contracted HUS. The investigation team identified flour as the culprit after much trial and error and identified O121 and O26 as the STEC serogroups. Studies such as this emphasize the importance of considering flour as a source of STEC contamination. Flour goes through no heat-kill steps during processing and has no bactericidal safety measures other than its inherent low-water activity. Therefore, it is important that flour is properly heat-treated during food preparation.

Foodborne Illness Outbreaks Related to *Salmonella*

In this section, some of the foodborne outbreaks linked to *Salmonella* that occurred in the US will be highlighted as documented by the CDC (CDC, 2024b). In September of 2018, the Kellogg's Brand Honey Smacks cereal was recalled due to *Salmonella* Mbandaka contamination. A total of 135 people were infected across 36

states, 34 were hospitalized but none died. In February of 2018 raw sprouts were associated with a *Salmonella* Montevideo infection. In total 10 people got sick across 3 states with no hospitalizations or deaths. Later in May of 2018, dried coconut of the International Harvest, Inc. brand Go Smiles was associated with *Salmonella* Typhimirium contamination. Across 8 states there were 14 cases reported with 3 hospitalizations, no deaths.

Later in October of 2020 there was a recall of Thomson International inc. onions due to *Salmonella* Newport infection. A total of 1,127 cases were reported across 48 states with 167 hospitalizations and 0 deaths. Then in July of 2022 a Jif brand peanut butter outbreak was caused by contamination of *Salmonella* Senftenberg. In this outbreak there were a reported 21 illnesses, 4 hospitalizations across 17 states and 0 casualties. In June of 2023, a Gold Medal flour outbreak due to *Salmonella* Infantis contamination occurred in the U.S. In this outbreak across 13 states there were 14 illnesses, 3 hospitalizations and 0 deaths. Later in July of 2023 there was a *Salmonella* enteritidis outbreak associated with Papa Murphy's cookie dough. The outbreak in 6 states resulted in 26 illnesses, 4 hospitalizations, and no deaths reported.

Foodborne Illness Outbreaks Related to Pathogenic *E. coli*

The CDC has summarized the recent *E. coli* outbreaks in the U.S. (CDC, 2024a). In June of 2009 Nestle Toll House refrigerated cookie dough was implicated in an *E. coli* O157:H7 outbreak. In this outbreak across 30 states 72 people were infected, 34 were hospitalized, 10 developed HUS, and zero died. Then in September of 2016 the STEC

O121 and O26 were implicated in a Gold Medal brand flour outbreak. The outbreak affected 24 states, 63 people were infected, 17 were hospitalized, 1 developed HUS and 0 died. Later in July of 2019 multiple brands and flour types were associated with STEC O26. In these outbreaks 21 people were infected with 3 hospitalizations across 9 states. Then in September of 2021 an outbreak of *E. coli* O121 was linked to cake mixes. In this incident 16 people got ill across 12 states, 7 were hospitalized and zero died.

Effect of Humidity on Baking

Humidity ratio is the mass of the water (in kg) divided by the mass of the dry air (kg) in an environment (Britannica, 2012). This is an important measurement because the more moisture that is present in the environment then the more efficient the baking process is (Science of Cooking, 2016). Not only does air conduct heat more efficiently with higher temperature (energy) air can conduct heat more efficiently, but products baked in higher humidity environments lose less water during the baking process. That is because in a closed system the humidity will eventually reach an equilibrium between the two mediums. This is a key factor to take into consideration while baking because it could be a confounding factor when comparing how two products bake. When humidity levels are high, dry ingredients such as flour, sugar, salt, and others will absorb more moisture and vice versa. Shrestha et al. 2016 demonstrated that maintaining an average of 20% relative humidity during baking enhances the total thermal lethality, compared to < 3%, while also maintaining quality.

Water Activity

Water activity (a_w) is the ratio of the vapor pressure of the food when compared to distilled water (FDA, 2014b). As a point of reference, distilled water has a a_w of 1.0, with everything else falling between 0 to 1. Many foods fall above 0.90 a_w, examples being fresh meat and fish (0.99), raw vegetables (0.99), raw fruits (0.98), and cooked meat and bread (0.91-0.98 a_w) (Manitoba Food Safety and Inspection Branch, 2024). Different kinds of bacteria and microorganisms require differing levels a_w to grow. For example, most gram-negative bacteria require at least 0.97 and most gram-positive bacteria require 0.90 while most yeasts require 0.88 and most molds require 0.80 a_w . It is worth noting that most mycotoxin producing fungi requires a_w greater than 0.85. A finished product is not subject to the regulations associated with emergency permit control, Thermally Processed Low-Acid Foods Packaged in Hermetically Sealed Containers, or acidified foods if its a_w is less than 0.85 (FDA, 2014b).The reason being that with the a_w so low there is no risk of bacterial growth occurring in said product, even if it contains few bacteria. Many bacteria can survive in low-water activity environments, even if they can no longer proliferate.

As observed above in the various *Salmonella* and pathogenic *E. coli* outbreaks and as mentioned by Beuchat et al. 2013, low water activity foods have recently been the source of an increasing number of foodborne illness outbreaks. According to Beuchat et al. (2013) a water activity of 0.87 is the approximate minimum requirement for bacterial growth, and therefore any food under that (either naturally, via drying or salt/sugar addition) will not allow bacterial growth. Although bacteria cannot grow, cells already present can survive with relative success, incubating until water is introduced to the

system either in food preparation or when consumed. Beuchat et al. (2013) documented that a very small number of cells of either *Salmonella* or pathogenic *E. coli* are able to cause infection. It has also been shown that these cells can survive for months or even years in these low water activity environments such as wheat flour (Beuchat et al., 2013).

Low Moisture Foods Challenges

The moisture content of the foods plays an important role in cooking or baking. The more moisture present in the food being cooked, the higher the relative humidity inside the oven. Furthermore, a higher humidity environment results in a slower formation of the baked goods' crust; and the slower the crust is formed the more consistent the heating will be throughout the baked goods (Science of Cooking, 2016). The union of all of this information results in foods with low moisture contents to be more problematic to heat or cook. These products dry out faster and don't heat as effectively at high-moisture levels due to reduced moisture available to produce steam. Another linked problem with this is case hardening.

Case hardening is when the outside of the food product sets in its shape and forms a crust around the product. In this case the outside gets hard and dry while the inside is still moist or dough-like in comparison (Gulati, 2015). Gulati also found that case hardening is positively correlated with higher drying/heating temperatures and is negatively correlated with relative humidity of the environment. As case hardening occurs and the water activity decreases on the surface but remains a higher moisture

inside the sample it is possible that the surviving bacteria on the surface will be more resistant to the heat because there is less moisture around them (Grasso-Kelley, 2020). In their study on hot air drying and apple pieces they found that case hardening did not impact microbial inactivation. However, they noted that was because they had already decreased microbial levels to below their detectable limit.

Bacteria will remain dormant if there isn't enough accessible water. Low moisture foods are an example of where bacteria may be present, but they aren't multiplying. Low moisture foods are those with a a_w equal to or less than 0.85 (Mermelstein, 2018). These foods won't support most bacterial propagation, including *Escherichia coli* and *Salmonella* with a minimum a_w requirement of 0.95 (Sperber, 1983). A baked good example would be shelf-stable soft cookies with an ideal water activity of 0.6 to 0.7 (Natacia, 2021). This water activity is too low to support bacterial growth but not too low for them to survive in. Xie et al. noted that the lowest water activity *Salmonella* can propagate in is 0.94 a_w, but they also mentioned it can remain viable at much lower levels for extended periods (Xie et al., 2021). It is possible that propagation may have occurred despite their final water activity not supporting microbial growth during batter preparation. Furthermore, even if the a_w doesn't support bacterial growth, if the heat-kill step was insufficient then the bacteria could be surviving in that low a_w environment for a large amount of time. A study on this was done comparing how *Salmonella* and *Escherichia coli* O121 survived in wheat flour over 360 days (Michael et al., 2022). In this study the *Salmonella* population started at 7.6 ± 0.18 log CFU/g, and *E. coli* started at 7.8 ± 0.07 log CFU/g. After 360 days *Salmonella* decreased to 2.0 ± 0.40 log CFU/g and *E. coli* decreased to 2.8 ± 0.59 log CFU/g. So, even after a year in the wheat flour at a

water activity ~0.65 both *Salmonella* and *E. coli* survived (Michael et al., 2022). In their research Michael et al. (2022) found that after 180-270 days the *E. coli* in storage had become acclimated to the environment and didn't decrease as much in this time period. Furthermore, *Salmonella* overall showed more thermal resistance than *E. coli* in this low water activity environment.

One of the primary concerns is with these pathogenic bacteria surviving inside these low water activity environments, such as flours and milk powders. There is a possibility that bacteria may multiply once the flour or milk powder is hydrated for food preparation. According to Sekhon (2020), even one cell of *Salmonella* can cause sickness, depending on the individual's health, age, and other factors. Bacterial resistance depends on many factors such as the water activity in the food, availability of food, the strain being tested, as well as the components that make up the food matrix. Fat content in food is known to increase the thermal resistance of *Salmonella* (Murphy, 2003). The pairing of a low/decreased water activity with foods containing high sugar, protein, or fat contents results in increased thermal resistance (Deák, 2014). This indicates that high concentrations of these components in a food matrix result in an overall higher heat resistance for bacteria. In fact, in *Salmonella* the *D*- values increased significantly as a_w decreases (Xie et al., 2021). Additionally, it was found that desiccation in oil (extreme drying) increases bacterial thermal resistance by decreasing the a_w of the bacteria (Yang, 2020). By decreasing/using a low a_w food matrix you are in turn decreasing the bacterial a_w, resulting in increased thermal resistance.

pH

pH measures alkalinity and acidity in a product based on its total level of hydrogen ions (the hydrogen potential) (Terra Food Tech, 2022). The pH scale goes from 0 to 14 with 0 being the most acidic, 14 being the most basic, and 7 being a neutral pH. Most foods trend towards acidity over alkalinity. As the article mentions, most foods fall between 1.5 to 10. Most bacteria prefer neutral conditions for growth, though some can tolerate lower pH levels to a certain degree. The industry food safety reference uses *Clostridium botulinum* as the target microbe as it is one of the most acid resistant bacteria that is also pathogenic and highly deadly. *Clostridium botulinum* cannot grow in pH levels lower than 4.6, so any food with a pH greater than 4.6 is termed a low acid food. Foods that are low acid need to be treated in another way to ensure they are safe to eat, such as via heat or water activity. Foods with a pH between 4 and 4.6 are termed acidic foods. Then at a pH of 4 or lower there are high-acid foods. Foods such as citrus fruits, cereals and yogurts fall in this category. While spores are not generated at this pH, other organisms such as yeasts, molds and acidophilic bacteria can still grow and spoil food (Terra Food Tech, 2022).

Importance of *D*- and *z*- values

The time required at a specific temperature to reduce the bacterial population by one log is known as the *D*- value whereas *z*- value is the temperature change required to increase/decrease a *D*- value by 1 log (FDA, 2014a). A *z*- value of a bacteria is found after multiple *D*- values have been calculated at varied temperatures for a specific food matrix. Meaning you could either raise or lower the temperature used in the heat-kill step

by the *z*- values temperature and the time required would be a log in difference (10-fold) to reach the same level of kill. Though the product must be specific because a multitude of factors influence the *D*- value of a specific bacteria including fat content, protein content, sugar content, water content, pH, etc. Additionally, even different strains of the same type of bacteria can have a varying *D*- values, therefore they must be specific to each bacterium and each matrix.

An important concept of microbial growth is that it is exponential (Ethide Laboratories, 2022). It is important to note that bacteria die at a constant rate, regardless of the total number present. This is what allows for *D*- and *z*- values to be useful for application in food industry and to optimize the kill step. Additionally, the initial amount of bacteria present is known as the bioburden. Therefore, the higher the bioburden, measured in Colony Forming Units (CFU), the longer/higher amount of heat-kill required.

The FDA and FSMA

As times changed and we learned more about foodborne illnesses and the world around us the FDA realized not enough was being done (FDA, 2024). With the implementation of the Food Safety Modernization Act, food safety as a whole has switched from a reactive to a proactive approach. It is estimated that every year 48 million people get sick, 128,000 are hospitalized and 3,000 die, all from foodborne illnesses (CDC, 2023). Statistics like this further accentuate the need for continued regulation and observation. Thanks to the FSMA regulations the FDA has gained much

more oversight into the food industry than they ever had before. They gained the ability to require companies to recall their products where it is deemed necessary, like with a foodborne pathogen outbreak. Before this act, the FDA could suggest recall, but ultimately it was the companies' choice. Additionally, the FDA gained the right to inspect company plants unannounced without needing for said companies' approval. The FDA has also required much more of a paper trail in these industries. With more documentation there is also more traceability, and ultimately it results in an overall more consistent and safer system. Thanks to improved communication between regulatory agencies such as the FDA and food companies' overall food safety has improved.

The FSMA "Final Rule for Preventative Controls for Human Food" is the reason for companies needing to prove their risk-based preventative control practices are meeting FSMA requirements (FDA, 2024). These preventative controls refer to their food safety plans and can range to anything relating to preventing hazards. Heat or thermal kill steps have always been important in food production. In this chapter, the focus will be on bakery validations performed to confirm thermal heat kill steps are adequate in eliminating potential foodborne pathogen assuring the safety of the finished food product.

Benefits to the Baking Industry

The more one understands about a process, technique, or organism the more appropriate one can respond to variations and problems that arise. You don't become an expert in something by assuming you have nothing to learn from others. The way you

become an expert and in turn stay on top in a field/subject is by taking in new information/research. These principles can be applied to the baking industry of today. Baked goods have been around for over 15,000 years. (Stafford, 2019). They started by using rocks as tools and an open fire to bake them. One can agree that baking has come a long way even from the Greeks and the Middle Ages. The techniques have changed because information was gained, and along with-it better techniques were adopted. The information gathered from baking validations provides the same potential for growth in the baking industry.

Through baking validations researchers are able to observe different heat kill steps in different product formulations. By testing specific products researchers are able to confidently provide the scientific documentary proof on how effective the said heat kill step is for the studied bacteria in that specific food matrix. By performing these product-pathogen specific validation studies researchers and industry can continuously improve their methods and understanding.

Baking validation plays an important role in optimizing the baking process, achieving food safety, and protecting consumers from foodborne illness outbreaks. A validation study provides scientific evidence that a process step is capable of controlling the identified biohazard (Ceylan et al., 2021). Additionally, by performing these validations food manufacturers are complying with FSMA by providing adequate documentation showing that their kill steps are efficient in controlling the potential foodborne pathogens (FDA, 2024). There are over 2000 bakery products on the market and the list is growing every year. Though it is a long process to test all products at risk of

pathogenic bacterial contamination, it is important to protect consumers and increase food safety.

An Overview of the Baking Validation Research Done to Date.

A strong focus on bakery validations in the research field has not been around for very long. One of the first validation studies was performed in 2014 on peanut butter cookies (Lathrop et al., 2014). This study demonstrated a 5-log reduction of *Salmonella* in peanut butter cookies in 14 minutes at 177°C (350°F). Two years later in 2016 Channaiah et al. performed baking validation research using hamburger buns (Channaiah et al., 2016). In this study again *Salmonella* was the bacteria of focus and a >5 log reduction was achieved in 6.0 minutes when baked at 218.3°C (424.94°F). In this study Channaiah also calculated the *D*- and *z*- values for *Salmonella* in hamburger bun dough. They found *D*- values of 28.64, 7.61 and 3.14 minutes at 55, 58, and 61°C respectively and a *z*- value of 6.58°C. Then in 2017 Channaiah published another research paper this time on plain muffins with *Salmonella* as the bacteria of focus (Channaiah et al., 2017). In this study they achieved a >5 log reduction in 17 minutes at 190.6°C (375°F). They found the *D*-values to be 62.2, 40.1 and 16.5 minutes at 55, 58 and 61°C respectively with a *z*-value of 10.4°C.

Then in 2018 Channaiah published another bakery validation research paper on Donuts using *Salmonella* (Channaiah et al., 2018). In this study the >5-log reduction was achieved in just 2 min at 190.6°C (375°F). The *D*-values were 8.6, 2.9, and 2.1 minutes at 55, 58, and 61°C respectively with a *z*-value of 10.0°C. In the following year Channaiah

published another bakery validation research paper over nut muffins inoculated via flour and an alternate inoculation via walnuts to control *Salmonella* (Channaiah et al., 2019a). In this study a >5 log reduction was found in both at 17 min at 190.6°C (375°F). The *D*-values of inoculated flour were 24.0, 4.0 and 0.6 minutes at 60, 65 and 70°C respectively with a *z*-value of 6.1°C. The inoculated walnut *D*-values were found to be 22.0, 3.6 and 1.7 minutes at 60, 65 and 70°C respectively and a *z*-value of 9.0°C. Later in 2019 Channaiah published another validation research paper using whole wheat multigrain bread (Channaiah et al., 2019b) to control *Salmonella* contamination. A >5 log reduction was found to be 15 minutes at 190.6°C (375°F). The *D*-values were 59.6, 20.0, and 9.7 minutes at 50, 52, and 55°C respectively with a *z*-value of 6.5°C.

In early 2020 Michael performed a study on pathogenic *Escherichia coli* 0121 in plain muffins in comparison to the previous study on plain muffins using *Salmonella* by Channaiah in 2017 (Michael et al., 2020). In this study the >5 log reduction was found at 17 min at 190.6°C (375°F). The *D*-values were 42.0, 38.4 and 7.5 minutes at 60, 65 and 70°C respectively with a *z*-value of 5.0°C. Later in 2020 Channaiah published a study on *Salmonella* and its survival in flour tortillas (Channaiah et al., 2021b). In this study a >5 log reduction was found after 60s at 221.1°C (430°F). The *D*-values found in this study were 22.2, 13.4, and 4.6 minutes at 55, 58, and 61°C respectively with a *z*-value of 8.9°C.

Then in early 2021 Unger published a paper on brownies focusing on two different types of bacteria, *Salmonella* and *Listeria monocytogenes* (Unger et al., 2021). The >5 log reduction was achieved by 40 min at 176.7°C (350°F). For *Salmonella,* the *D*-values were found to be 53.4, 27.2 and 10.7 minutes at 64, 68 and 72°C respectively with

a *z*-value of 11.1°C. Then for *Listeria monocytogenes* the *D*-values were found at 37.5, 16.9, 9.1 minutes at 64, 68 and 72°C respectively with a *z*-value of 16.4°C. Then in late 2021 Channaiah published a paper comparing *Salmonella* survivability in hard and soft cookies (Channaiah et al., 2021a). For soft cookies, the >5 log reduction was achieved by 11.5 min at 185°C (365°F) with hard cookies being 20.5 min at 185°C (365°F). The *D*-values for soft cookies were found to be 62.3, 28.6 and 14.4 minutes at 60, 65 and 70°C respectively with a *z*-value of 15.8°C. The hard cookies *D*-values were found to be 59.6, 28.1 and 11.9 minutes 60, 65 and 70°C respectively with a *z*-value of 14.5°C. Singh & Channaiah studied the traditional crust pepperoni pizza to control *Salmonella* contamination and reported a >5 log reduction when the pizza was baked at 260°C (500°F) for 12 min (Singh & Channaiah, 2022). The *D*-values were found at 49.5 ± 4.10 15.3 ± 0.68 and 2.8 ± 0.31 minutes at 55, 58 and 61°C respectively with a *z* value of 4.8 ± 0.16°C.

As one can see, the research has evolved over time, presenting more and more useful data as the methods are better streamlined and understood. This is just the start; more product-pathogen specific validation studies need to be done to further improve food safety and to gain better understanding of how these pathogenic bacteria behave in our food products. All of the research previously described is summarized in **Table 1**.

CHAPTER 2

VALIDATION OF A SIMULATED COMMERCIAL BAGEL BAKING PROCESS TO CONTROL *SALMONELLA* CONTAMINATION

Introduction

Safe food is one of the basic necessities of life for good health and well-being. However, in today's fast-paced world, it isn't practical for everyone to prepare their own food for the day. By placing such an important part of their existence in the hands of others, consumers are putting their trust in food manufacturing companies to ensure that the food they are providing is safe. This is a significant issue as nearly 48 million people get sick from foodborne illnesses every year (CDC, 2023). Of those 48 million, around 128,000 people end up in the hospital and nearly 3,000 die every year. Food manufacturers and distributors have an obligation to provide food that is safe and free of biological, chemical, and physical contaminants. One of the most comprehensive forms of regulations was the Food and Drug Administration's (FDA) Food Safety Modernization Act (FSMA) that was passed in 2011. (FDA, 2024). Under this law, the FDA was granted more authority including the ability to require a company to recall a food product and perform inspections on their own terms. This has changed their food safety oversight from a reactive stance of mitigating damage to a proactive stance where they had much more influence. Another important part of the FSMA act was mandating companies to implement validated preventive controls to control the potential hazard

assuring the safety of the finished food product. According to the FDA's FSMA validation requirement (§ 21 CFR 117.160), the food manufacturers must validate that the preventive controls identified and implemented in accordance with § 117.135 are adequate to control the hazard as appropriate to the nature of the preventive control and its role in the facility's food safety system (FDA, 2023a). The FDA has made validation mandatory for all food manufacturing businesses that must register with the FDA. This has created an industrywide need to validate all preventive controls such as baking, cooking, extrusion, boiling, roasting etc., assuring food safety.

Bagels are one of the most popular breakfast and brunch menus around the world (Manzoor et al., 2024). Although bagels and bread both have similarities, one major difference is that bagels are boiled before baking, while bread is not boiled (Jorge, 2024). The boiling process allows the bagels to gelatinize the starches on the bagel's outer surface, revealing the crunchy exterior. Typically, bagels are made from a dough made up of flour, yeast, salt, and a sweetener which is then shaped by hand into a distinctive ring or torus shape, briefly boiled in water, and then baked (Jorge 2024). It is estimated that 61% of adults in the United States consume bagels (Kenney, 2014). That is over 150 million people that do or have consumed this product. Additionally, as a very popular breakfast food, it can be eaten as the base for a sandwich, plain, or with spreads such as cream cheese. The largest producer of grocery store sold bagels is Thomas Bagels where in 2014 they sold over 160 million bagels (Manufacturing.net, 2015). Moreover, traditional sized plain bagels were among the top 5 most popular bagels. The traditional-sized plain bagels will be the focus of this study.

Salmonella is a Gram-negative bacterial pathogen responsible for ~200 million to over 1 billion infections worldwide, with 93 million cases of gastroenteritis and 155,000 deaths with 85% of illnesses being food-linked (He et al., 2023). Not only is it the 2nd highest cause of foodborne illness in the US, but it is also the top cause of both hospitalizations and deaths in the US (CDC, 2023). *Salmonella* can be found in all sorts of food products from fresh fruits and vegetables to unpasteurized dairy products, to undercooked meats (Partnership for Food Safety and Education, 2024). In addition to those products, *Salmonella* has also been known to survive in low water activity environments such as flour for extended periods (Forghani et al., 2019). It was also found that due to a low water activity environment *Salmonella* can gain increased heat tolerance, raising its survivability in low water activity foods (Gruzdev, 2011). In 2023 a multistate outbreak of Gold Medal brand flour resulted in 14 illnesses across 13 different US states (FDA, 2023c). This clearly shows that although *Salmonella* cannot multiply in low-water-activity foods such as wheat flour, however it can survive for weeks and even months.

To the best of the authors' knowledge, there have been no prior studies on thermal inactivation of *Salmonella* in plain bagels. Therefore, a study was conducted to validate the effectiveness of plain bagel baking process as an effective kill-step for controlling *Salmonella* in the event of pre-baking contamination. The main objective of this study is to validate a simulated commercial bagel baking process (232°C (450°F) for 14 min) to control *Salmonella* contamination. Additionally, the thermal inactivation parameters (*D*- and *z*-) of *Salmonella* bagel dough, pH and water activities of the bagel during baking were also determined in plain bagel.

Materials and Methods

Experimental design -

Two independent studies were performed to validate plain bagels to control *Salmonella* contamination and to determine its heat resistant characteristics in plain bagel dough. The first study was performed to validate the plain bagel baking process to control *Salmonella* that originated from flour. The second study was conducted to determine the heat resistance characteristics (*D*- and *z*- values) of *Salmonella* in plain bagel dough. During the baking process plain bagels samples were collected at regular intervals to determine the a_w and pH of the plain bagels. In this experiment we employed a completely randomized block design consisting of 9 sample points *viz.*, pre-proofing, post-proofing/0, 1 min of boiling, 2 min of boiling, 3.5 min of baking, 7 min of baking, 10.5 min of baking, 14 min of baking as well as 15 min of post baking ambient air cooling. Three independent replications were performed, and one-way ANOVA was performed using Minitab® 19 to determine statistical differences (α=0.05) among treatments.

A randomized complete block design (replications as blocks) was performed for *D*- and *z*- value determination with three replications. The *D*- values for the *Salmonella* cocktail were determined at temperatures 56°C, 59°C and 62°C. The linear regression graphs were plotted using Microsoft® Excel 2024 and the statistical differences in the *D*- and *z*- values were determined using one-way ANOVA using the statistical software Minitab® 19.

Microbial Cultures -

In this study we utilized 7 serovars of *Salmonella*, all of which were purchased from the American Type Culture Collection (ATCC®; Manassas, VA, USA). The *Salmonella* cultures were selected based on heat resistance as well as their association with food-borne illness outbreaks. Details of *Salmonella* serovars can be found in **Table 2**. *Salmonella* serovars were stored at -20°C in Brain Heart Infusion (BHI) broth. Before use, all *Salmonella* serovars were propagated in BHI broth and stored at 4°C.

Master inoculum preparation -

For each replication 0.1 ml of each stock culture was added to 10 ml of BHI broth and incubated at 37°C for 24 hours. After incubation 250 µl of a given serovar were pipetted onto BHI plates and spread plated. This was done for all the *Salmonella* serovars before incubating the plates at 37°C for 24 hours. After the incubation, plates containing *Salmonella* cells were harvested with (2 applications) 1 ml autoclaved DI water and colonies were dislodged with L-spreaders where the inoculum was added to sterile 10 ml centrifuge tubes. An individual tube was used for each serovar. Once all the *Salmonella* serovars were harvested, equal portions of each serovar's tube were combined in a sterile 50 ml centrifuge tube to obtain a 7-serovar *Salmonella* cocktail inoculum. This master inoculum was then used for flour inoculation.

Flour inoculation -

The flour inoculation with the *Salmonella* cocktail was performed as previously described by Channaiah et al. (2016). Per replication 400g of King Arthur® unbleached bread flour was weighed and added to a sanitized plastic tub (~38 × 26 × 7 cm, Rubbermaid Inc., Huntersville, NC, USA). A spray nozzle calibrated to spray 1 ml per spray was sanitized and attached to a nozzle of the 50 ml master inoculum centrifuge tube. The flour was mist inoculated with 8 ml of the master inoculum in a Class II, Type A2 Biological Safety Cabinet (LabConco Corporation, Kansas City, MO, USA). Afterwards, the flour was thoroughly mixed, and all the flour chunks were broken up and dispersed. Later the inoculated flour bin was incubated at 37°C (±1°C) for ~7 hours until the a_w reached pre-inoculation levels. Once dried, the inoculated flour was sealed in the airtight container and stored at room temperature (~21°C) and used within 2 days (Channaiah et al., 2016).

For the *D*- and *z*- value study 200g of unbleached bread flour was weighed and added to a sanitized tub. The sample was mist inoculated as previously described where 4 ml of the *Salmonella* master inoculum was added. The flour was then mixed and dried as previously mentioned.

Bagel dough preparation -

The plain bagel recipe as well as the baking parameters were given by American Institute of Baking International (AIB International, Manhattan, KS, USA). All ingredients used in this study were purchased from a local grocery store in Columbia,

MO. The inoculated flour was weighed and added to a sanitized mixing bowl where flour, sugar, salt, dry yeast, and water were all added in their respective weights. Refer to **Table 3** for the plain bagel recipe. Afterwards everything was mixed in a KitchenAid (Benton Harbor, MI, USA) stand mixer with a dough hook for 7 min at low speed until the dough had formed. Once the dough was formed it was separated into twelve 85g dough balls and two 30g dough balls. The 85 g of dough balls were then rolled and coiled into a circle (~21cm long) where water was added to one end to aid in adhesion. The 30g dough balls were rolled and one was taken as the pre-proof sample. Then the 12 bagels and the 30g of dough were placed on a metal cookie sheet with parchment paper, covered in polyethylene wrap and proofed at 37°C for 25 min.

For the *D*- and *z*- value study (for recipe please refer to **Table 4**) the yeast was removed. as it could lead to gas expansion in the Thermal Death Time (TDT) disks which could prevent opening the disks. Once the ingredients were mixed (as previously described) polyethylene wrap was used to cover the metal bowl and the product was proofed at 37°C for 25 min.

Plain bagel dough boiling and baking -

After proofing, bagels were added to a metal cage (13.25 in by 8 in) to be boiled in a pan (14 in by 4 in) on the stovetop. Before boiling, a 30g sample was taken as the post proof (0-min sample). Five K-type thermocouples were randomly inserted into the geometric center (cold spot) of bagels with the sixth one measuring the temperature of the boiling water. Later these thermocouples were attached to a 6-channel data logging

system (SuperM.O.L.E.® Gold 2, ECD, Milwaukie, OR) which measured the temperature every second. The plain bagels were allowed to float in the boiling water for 1 min and then flipped with metal tongs after 1 min. At the 1 min boiling mark, a sample was taken for *Salmonella*, pH and a_w analysis. Following 2 min of boiling, all samples were removed from the boiling water, and one sample was taken at that time. The bagels were allowed to rest for 2.5 min ±30s on a cooling rack before being placed into the 232°C (450°F) oven on a metal rack. Subsequently, samples were collected randomly every 3.5 min for further analysis. After 14 min of baking, a sample was taken and the rack of bagels was removed and allowed to cool for 15 min at ambient air cooling before the final sample was taken. During baking, the oven door was open for less than 10s at each sampling point (Channaiah et al., 2016) to collect plain bagel samples.

Water activity (a_w), pH and humidity ratio -

The a_w of plain bagel samples were measured at 25°C with the Novasina Labswift Portable water activity meter (Novasina-AG, Lachen, Switzerland) as per the method described by Michael et al. (2020). The a_w and pH samples were measured at pre-proof, post-proof/0, 1 min boil, 2 min boil, 3.5 min bake, 7 min, 10.5 min, 14 min and after 15 min of ambient air cooling. At 3.5 min of baking bagel a_w samples were determined in crumb (the geometric center portion of the dough/bagel) and the crust (the outermost part (1-3mm of crust) of the bagel. The reason for this was the formation of a clear crust as baking progressed. The plain bagel samples for a_w were placed into water activity cups and were capped and then measured once cooled. A pH meter (FiveEasy F20 pH meter, Mettler-Toledo, Greifensee, Switzerland) was used to measure the plain bagel samples

after the bags containing plain bagel samples were stomached for 1 min at 30 rpm. In a separate study a Scorpion® 2 Digital Humidity Sensor (Reading thermal, Sinking Spring, PA, USA) was used to measure the humidity inside the oven during the baking process. This study was performed in triplicate wherein the sensor was placed inside the oven along with bagels during baking at 450°F for 14 min. The relative humidity ratio (kg of moisture / kg of dry air) was recorded every second during the bagel baking.

D- and *z*- values determination -

A 7-serovar *Salmonella* cocktail containing ~10 logs was used to carry out the *D*- and *z*- value studies. The target temperatures for *D*- value determination were 56°C, 59°C and 62°C as described by Channaiah et al. (2019b). In this study a sanitized and temperature-controlled water bath (Precision CIR19, Thermo Fisher Scientific, Newington, NH, United States) was filled with DI water and ~100 floating plastic balls were used to maintain the water temperature. The TDT disks were used to hold dough samples and keep the samples dry (Engineering Shop, Washington State University, Pullman, WA, USA). The height of the TDT disks was 5mm and the diameter of the disks were 50mm (internally). A total of 10g of inoculated dough was added to each TDT disk (4 regular and 1 with a T-type thermocouple to measure internal temperature of the disk). Samples were sealed inside the disks and added to the water baths in sets of 5 for each of the three temperatures (in three replications) studied. Once the internal temperature of the disk reached the target temperature, the first sample (the 0) was taken. For 56°C, samples were taken at 0, 25, 50, 75 and 100 min. For 59°C, samples were taken at 0, 12, 24, 36 and 48 min. Then for 62°C, samples were taken at 0, 2, 4, 6 and 8

min. Once each sample was removed from the water bath it was immediately placed into ice water to arrest any further heat-kill effect. Temperatures in the disks were monitored using a digital thermometer (Fluke 51–2 Thermometer, Everett, WA, USA) connected to the TDT disk.

Microbial (*Salmonella*) Analysis -

The *Salmonella* enumeration was performed as described by (Channaiah et al., 2016). Samples were taken at: pre-proof, post-proof/0, 1 min boil, 2 min boil, 3.5 min bake, 7 min, 10.5 min, 14 min and after 15 min of ambient air cooling. A 30g portion of each sample was added to stomacher bags containing 50 ml of chilled 0.1% peptone water and stomached at 300 rpm (Stomacher® 400 Circulator, Worthing, West Sussex, United Kingdom) for 1 min before being refrigerated at ~4°C and analyzed within 1 hour. Samples were then serially diluted in 9 ml of 0.1% peptone water and plated on BHI agar (nutrient rich media). Plates were then inverted and incubated at 37°C for 4 hours. After 4 hours the plates were removed from the incubator and overlaid with ~15 ml of Xylose Lysine Deoxycholate (XLD) agar. Once the agar had solidified, the plates were again inverted and incubated at 37°C for another 20 hours (Singh et al., 2022).

For *D*- and *z*- value analysis, after samples were cooled to ~4°C, the plain bagel dough samples were added to chilled stomacher bags containing 10 ml of 0.1% peptone water and refrigerated at ~4°C and analyzed within 1 hour. Samples were then serially diluted in 9 ml of 0.1% peptone water and plated on BHI agar. Plates were then inverted and incubated for 4 hours until they were overlaid with the selective media XLD agar and

incubated for 20 hours. After the incubation, colonies were enumerated with a Reichert Quebec Darkfield Digital Colony Counter-110 V (Reichert, Inc., Depew, New York, USA) to enumerate the *Salmonella* cells. The *Salmonella* cells were enumerated as colony forming units (CFU/g).

Results:

For the plain bagel validation study, the seven serovar *Salmonella* cocktail master inoculum contained a total of 11.0 ±0.18 log CFU/g while the flour retained a 8.1 ± 0.47 log CFU/g. The plain bagel validation study resulted in a reduction of 6.3 ±0.799 CFU/g in *Salmonella* (**Figure 3)**. A significant reduction of >5 log CFU/g in the *Salmonella* population was observed after 7.0 min of baking. During boiling, the maximum temperature recorded after 2 min of boiling was 105.5 ±3.8°C (**Figure 4**). During the 14 min of baking validation study, the maximum internal temperature of plain bagel was 194.1 ±0.5°C, while immediately after the 15 min of ambient air cooling the mean internal temperature of plain bagel was 124.7 ±2.0°C (**Figure 5**). **Figure 6** shows the mean humidity ratio in the oven (0.01039 ±0.00241 kg moisture/kg air) at the start of baking which reached 0.43848 ±0.00770 kg moisture/kg air at the end of baking. Then **Figure 7** shows the final bagel product after boiling, baking, and cooling.

The a_w of the crumb portion of the plain bagels ranged from 0.928 ±0.002 to 0.943 ±0.008 (**Figure 8**). The a_w of the crust (external) portion of the bagels ranged from 0.823 ±0.005 to 0.928 ±0.005. There were no significant differences in the a_w of the crumb portion of the bagels. The a_w of the crust at 14.0 min of baking was significantly

different than all other a_w samples. Likewise, the a_w of crust samples at 10.5 min and 29.0 min were also significantly different from all others. Except for the pre-proof and 7.0 min bake samples, the a_w of 7.0 min crust sample was significantly different from every other crumb sample. The pH of plain bagels ranged from 5.21 ±0.015 at pre-proof to 5.94 ±0.217 at the end of 15 min of ambient air cooling (**Figure 9)**. There was a significant difference between the pH of pre and post proof samples with every sample after 2.0 min of boiling. Additionally, the pH of plain bagel at 1 min boil sample was significantly different from every sample after 3.5 min of baking.

The 7 serovar *Salmonella* master inoculum used for *D*- and *z*- value study contained 11 ±0.0 log CFU/g. However, the flour and dough retained 7.1 ±0.2 and 6.9 ±0.2 log CFU/g respectively. The *D*- values of the seven serovar *Salmonella* cocktail were calculated by graphing the log CFU/g against the 3 temperatures (°C) used in this study. The 7 serovar *Salmonella D*- values were 24.5 ±1.50 min (56°C), 13.3 ±1.85 min (59°C), and 6.8 ±1.05 min (62°C). **Figure 10** shows the graph used to calculate the *D*-values of the 7 serovar *Salmonella* in plain bagel dough. Furthermore, the log of those *D*-values were plotted against temperature (°C) to determine the *z*- value (11.1 ± 1.58°C) of the 7 serovar *Salmonella* cocktail (**Figure 11**). Then **Table 5** lists the calculated *D*- and *z*-values.

Discussion

Salmonella has been linked to numerous foodborne illness outbreaks, specifically in bakery and low- a_w related products. As previously mentioned, the average gram-negative bacteria requires a 0.97 water activity to be able to grow and multiply (FDA,

2014b). However, according to reports, *Salmonella* can thrive at water activity as low as 0.93 (Albrecht, 2015). It is possible for *Salmonella* to grow in bagels (the water activity of crumb is >0.93) in case of an improper boiling and baking. As far as pH is concerned, it rose slightly during the boiling and baking processes. But the final pH was well within the optimum pH range for *Salmonella* growth of 4.1-9.0 (Albrecht, 2015).

In this study the bagel baking process demonstrated a >5 log CFU/g reduction in *Salmonella* population by 7.0 min of baking. The limit of detection in this study was 0.4 log CFU/g. Earlier researchers also reported similar trends. Lathrop et al., 2014 demonstrated a >5 log CFU/g reduction in *Salmonella* population in peanut butter cookies after 13 min of baking at 350°F (177°C). In a similar study Channaiah et al., (2019b) validated whole wheat bread inoculated with *Salmonella* via flour. In this study they were able to achieve a >5 log CFU/g reduction after 15 min of baking at 375°F (190.6°C). Here, the bread pH was similar to that found in bagels but the a_w was high (0.97) in the crumb portion of the bread throughout the baking. It is possible that a higher kill was achieved in the whole wheat bread due to the increased bake time.

The *D*- values of 7 serovar *Salmonella* in plain bagels were 24.5 ±1.50, 13.3 ±1.85, and 6.8 ±1.05 min at 56, 59, and 62°C, respectively. The *z*- value of 7 serovar *Salmonella* in plain bagels was 11.1 ±1.58°C. Earlier research reported a similar trend as well. Channaiah et al., (2019b) studied *Salmonella* in multigrain bread and found *D*-values of 59.6, 20.0, and 9.7 min at 50, 52, and 55°C temperatures , respectively with a *z*-value of 6.5°C. These values indicate that bagels are a more heat resistant food matrix than that of a whole wheat multigrain bread, demanding a higher temperature to reduce the *Salmonella* in case of pre-baking contamination. In another study, Moura-Alves et al.,

(2020) determined the *D*- and *z*- values in egg inoculated *Salmonella* pastries. In their study, they found *D*- values of 53.19, 20.4, 6.95, and 1.60 min at, 52, 55, 58, and 61°C, respectively with a *z*- value of 5.96°C. Comparing these results to the bagels inoculated with *Salmonella* again clearly demonstrates that bagels appear to be a very heat resistant food matrix. Also, this could be attributed to flour being the source of *Salmonella* inoculation compared to eggs, because in flour the bacteria have had more time to adjust to harsh low water activity conditions, possibly increasing their thermal resistance. The different *D*- and *z*- values determined in this study could be caused by the thermal resistance differences between different *Salmonella* serovars in different matrices.

Conclusion

This study validated that baking plain bagels at 450°F for at least 14 min will result in a >5 log reduction in *Salmonella* population, thus providing an effective kill-step in case of pre-baking contamination.

Additionally, the *D*- and *z*- values determined in this study could be useful for developing predictive lethality models to calculate the total process lethality for *Salmonella* in plain bagel matrix. It is worth noting that the *D*-and *z*- values calculated here are specific to the bagel recipe used in this study. Any addition or retraction from this recipe would warrant a separate study to determine the new *D*- and *z*- values associated with that product due to the varying intrinsic and extrinsic factors.

CHAPTER 3

VALIDATION OF A SIMULATED BAGEL BAKING PROCESS TO CONTROL SHIGA TOXIN PRODUCING *ESCHERICHIA COLI* CONTAMINATION

Introduction

The majority of foodborne illnesses are due to infectious or toxic substances produced by bacteria, viruses, parasites, or chemical substances that enter the body through contaminated food products (WHO, 2022). An estimated 600 million people in the world fall sick after eating contaminated food and ~ 420,000 die every year (WHO, 2022). Additionally, these outbreaks undermine the confidence of food manufactures leading to a loss of US $110 billion each year in productivity, recall and medical expenses (WHO, 2022). One of the top foodborne pathogens among bacteria, pathogenic *Escherichia coli*, is responsible for producing Shiga toxin and affects millions of people annually, sometimes leading to severe and fatal outcomes (WHO 2022, CDC 2023). The STEC are *E. coli* that produces a toxin (Shiga toxin) that can result in serious illness via consumption of the contaminated food products (California Department of Health, 2023). According to the National Institute of Health (NIH), STEC annually causes 2,801,000 acute illnesses, 3,890 hemolytic uremic syndrome (HUS) cases, and 230 deaths globally (Majowicz et al., 2014). Not only can pathogenic *E. coli* be found raw and undercooked meats, raw vegetables, and unpasteurized milk products, but it can also be found in manure and in bodies of water where it's been found to survive for months (WHO, 2018)

contaminating the agriculture products at the farm level. A number of STEC infection outbreaks associated with dry foods and ingredients have been reported worldwide in the last two decades. In 2009, 80 people were reported to have been infected with *E. coli* O157:H7 infections caused by eating commercially manufactured, refrigerated, raw cookie dough in multiple states (Neil et al., 2012). In another incident, 21 people in 9 states were infected with *E. coli* O26 in 2019 due to contamination of flour in multiple cake and brownie mixes (CDC, 2019).

With this many people being impacted: it is clear that stringent food safety regulations are required to enhance food safety. The Food Safety Modernization Act (FSMA) enacted in 2011, was specifically designed to strengthen the food safety parameters in a farm-to-fork model, thus protecting the consumers from foodborne illness outbreaks (FDA, 2024). This legislation changed a reactive system to a proactive system by granting additional power and authority to the FDA. Some of these include the ability to inspect facilities at their own discretion, the ability to force companies to recall products when necessary, and most importantly it required food manufacturers to provide validation documentation as a scientific proof for their preventative control practices and how they ensured food safety in their products.

A bagel is a doughnut-shaped yeast-leavened bread roll that is characterized by a crisp, shiny crust, a dense interior, and a hole in the middle. The bagel has its roots in Jewish cuisine (origins dating back to the 17th century in Poland) and has become a staple in breakfast and brunch menus around the world (Jorge, 2024). In 2020 an estimated 202 million Americans ate bagels at least once (Statista, 2024). The major ingredients used in

bagel manufacturing are flour, yeast, salt, and sweetening. With over 60% of the US population consuming bagels, it highlights the importance of bagel manufacturing producers. This study focuses on standard plain bagels as they are one of the top 5 most popular bagels and are of a good standard (Manufacturing.net, 2015). To our knowledge, there have been no prior studies on validation of plain bagel baking to control STEC and heat resistance characteristics of STEC in plain bagels. Therefore, a study was conducted to validate the effectiveness of the baking process (450°F for 14 min) as a kill-step for controlling potential STEC contamination during plain bagel manufacturing and to determine the thermal inactivation parameters (*D*- and *z*-) of STEC in plain bagel dough. Additionally, the pH and water activity (a_w) in bagel dough were also determined.

Materials and Methods

Experimental Design -

Two separate studies were conducted to validate the plain bagel baking process to control STEC contamination and determine the heat resistance characteristics (*D*- and *z*-values) of STEC in plain bagel dough. The first study focused on validating a simulated bagel baking process where STEC was introduced via flour. Here the bagels were boiled for 2 min, flipping at 1 min, and baked at a temperature of 232°C (450°F) for 14 min followed by 15 min of ambient air cooling. There were a total of nine sampling points *viz.*, pre-proof dough, post-proof dough/0, 1 min of boiling, 2 min boiling, 3.5 min baking, 7 min baking, 10.5 min baking, and 14 min followed by a final sample taken after 15 min of ambient air cooling. Additionally, the a_w and pH were determined at each of

these sampling points. This study employed a randomized complete block design with replications as blocks. Three independent replications were conducted, and the statistical significance was determined via one-way ANOVA using Minitab®.

The second study focused on determining the heat resistance characteristics (*D*- and *z*- values) of STEC in bagel dough. For this we employed three temperatures *viz*., 56°C, 59°C and 62°C with the respective times of 0, 25, 50, 75 and 100 min, 0, 12, 24, 36 and 48 min and 0, 2, 4, 6 and 8 min. Microsoft® Excel 2024 was used to plot the linear regression graphs and statistical differences in STEC's *D*- and *z*-values were determined using one-way ANOVA using Minitab® 19 as the statistical software.

Microbial (STEC) Cultures -

A total of five STEC strains were used in this study. Three cultures were obtained from the American Type Culture Collection (ATCC®; Manassas, VA, USA). One from the United States Department of Agriculture (USDA) and another from the Center for Disease Control and Prevention (CDC). Please refer to **Table 6** for details. The STEC strains selected in this study were based on their associated foodborne illness outbreaks and/or food recalls. The STEC strains were stored in Brain Heart Infusion (BHI) broth and stored at -20°C. Before use, the STEC cultures were propagated in (BHI) broth and then stored at 4°C.

STEC Master Inoculum Preparation -

To prepare the STEC master inoculum, BHI agar plates were inoculated with 250 µl of each respective strain in duplicate (for a total of 10 plates). After the inoculation, each plate was spread-plated and then inverted and incubated at 37°C for 24 hours. Afterwards, the STEC plates were harvested by adding a total of 2 ml of autoclaved DI water per plate where the STEC cells were dislodged using an L-spreader. The respective STEC strain along with the DI water were then transferred to sterile 10 ml centrifuge tubes (separate tubes). Once all strains were harvested, inoculum from each strain were then evenly combined into one 50 ml centrifuge tube to form a 5-strain STEC cocktail. The STEC cocktail was used to inoculate the flour used to prepare plain bagels.

Flour Inoculation -

The flour inoculation was carried out as previously described (Channaiah et al., 2019b). A total of 400g of King Arthur® unbleached bread flour was added to a sanitized plastic tub (~38 × 26 × 7 cm, Rubbermaid Inc., Huntersville, NC, USA). A spray bottle nozzle calibrated to mist spray ~1 ml was attached to the 50 ml centrifuge tube containing the master inoculum. In a Class II, Type A2 Biological Safety Cabinet (LabConco Corporation, Kansas City, MO, USA) a total of 8 ml of inoculum was mist inoculated into the flour tub. The flour was then thoroughly mixed, where all chunks were broken up for uniform distribution of the STEC cells in the flour. Later the flour was dried in an incubator set at 37°C (±1°C) for ~6 hours until the a_w level dried back to its pre-inoculation level. Once dried, the flour was then sealed in a plastic container and kept at room temperature (~21°) until used within 2 days.

For the *D*- and *z*- value trials 4 ml of STEC inoculum was mist inoculated into 200g of flour, dried back to its pre-inoculation a_w levels and stored at room temperature (~21°C) until use within 2 days.

Plain Bagel Dough Preparation -

The plain bagel recipe and the baking parameters were given by American Institute of Baking International (AIB International, Manhattan, KS). All the ingredients used in this study were purchased from a local grocery store in Columbia, MO. Please refer to **Table 7** for the bagel recipe. All ingredients were added to a sterilized metal bowl and then mixed at low speed for 7 min using a dough hook in a stand mixer (KitchenAid, Benton Harbor, MI, USA). Later, the dough was then weighed into two 30g dough balls and twelve 85g dough balls. The 85g dough balls were then rolled out into ~21 cm in length and formed into the signature ring shaped bagel with a hole in the center. One 30g dough sample was rolled out and then taken as the pre-proof sample. The bagels were transferred to a cookie sheet with parchment paper, wrapped in plastic, and proofed for 25 min at 37°C. After proofing the second 30g sample was taken as the post-proof/0 sample.

For the *D*- and *z*- value study, the inoculated dough was prepared as previously described but without yeast and was wrapped in a polyethylene covered metal bowl and proofed at 37°C for 25 min. Yeast was excluded from the formulation to prevent expansion of dough as well as formation of air pockets which will affect the temperature reading in the TDT disk (thus preventing them from opening). Please refer to **Table 8** for

the recipe used to prepare plain dough for the *D*- and *z*- value study. Post-proofing, 10g of dough were then added to Thermal Death Time disks (TDT) to determine the *D*- and *z*- values of STEC in bagel dough.

Bagel Boiling and Baking -

A 6-channel data logging system (SuperM.O.L.E.® Gold 2, ECD, Milwaukie, OR, USA) was used to measure the internal temperature of the bagels (every 1 second) during boiling and baking. For this, five K-type thermocouples were inserted into the geometric center (as cold points) of the bagels randomly and the sixth thermocouple was used to measure the boiling water and/or oven air temperatures. All the bagel-doughs were placed into a (13.25 in by 8 in) metal cage and lowered into a pot (measuring 14 in by 4) containing boiling water. The bagels were boiled for one min (making sure they weren't stuck to the bottom of the cage) and then flipped. The 1 min boil sample was taken at this time. The bagels were boiled for an additional min and then all samples were removed from and placed onto a metal rack to rest for 2.5 min ±30s and the 2 min boil sample was taken at this time. Then bagels were placed into the oven and baked at 232°C (450°F) for a total of 14 min. Here, bagel samples were collected at 3.5 min, 7 min, 10.5 min, and 14 min for further analysis. Once the bagels were removed from the oven another sample was collected after 15 min of ambient air cooling.

Water Activity (a_w), pH and Humidity ratio -

During the plain bagel baking process, the a_w was measured at each subsequent sample time as described by Channaiah et al. (2021a). For a_w, the plain bagel samples were placed into sterile water activity cups, capped, and analyzed using a Novasina Labswift Portable water activity meter at 25°C (Novasina-AG, Lachen, Switzerland). After 3.5 min into the baking a noticeable crust formation was observed and two separate a_w readings were collected for the crust and crumb potions of the bagels. A pH meter (FiveEasy F20 pH meter, Mettler-Toledo, Greifensee, Switzerland) was used to measure the pH of each plain bagel sample. The humidity inside the oven during baking was measured in a separate study using a Scorpion® 2 Digital Humidity Sensor (Reading thermal, Sinking Spring, PA, United States). In this triplicate study the humidity ratio (kg of moisture / kg of dry air) inside the oven during bagel baking was measured by placing the sensor inside the oven along with the bagel dough.

D- and *z*- Values Determination -

For *D*- and *z*- value determination, the STEC master inoculum (~9 logs) was used to prepare the plain bagel dough. All five TDT disks (each containing 10g plain bagel dough) and a T-type thermocouple (attached to one disk for internal temperature monitoring) were transferred to preset hot water baths at specified temperatures. The temperatures used in this study were 56°C for 0, 25, 50, 75 and 100 min, 59°C for 0, 12, 24, 36 and 48 min and 62°C for 0, 2, 4, 6 and 8 min. The TDT disks were placed into sterilized water baths (Precision CIR19, Thermo Fisher Scientific, Newington, NH, USA) heated to the specified temperatures and monitored in real time using a K-type thermometer (Fluke 51–2 Thermometer, Everett, WA, USA). Samples were placed into

the water bath in sets of 5 and the first sample (0 sample) was removed once the internal temperature of the disk reached the above target temperature. Once removed, the samples were immediately placed into an ice-water bath to prevent further thermal-killing.

Microbial (STEC) Enumeration -

The enumeration of the STEC were carried out as previously described (Channaiah et al. (2017). Samples collected at pre-proof, post-proof/0, 1 min boil, 2 min boil, 3.5 min bake, 7 min, 10.5 min, and 14 min as well as 15 min after ambient air cooling were analyzed for STEC survivability. Plain bagel samples (~30g of each sample) were placed into stomacher bags containing 50 ml of sterile 0.1% peptone water and stomached (Stomacher® 400 Circulator, Worthing, West Sussex, United Kingdom) for 1 min at 300 rpm. After stomaching, the plain bagel samples were serially diluted in 9 ml of sterilized 0.1% peptone water and plated onto BHI agar. Plates were then inverted and incubated at 37° for 4 hours where they were then overlaid (~15 ml) with the selective media MacConkey Agar where they were allowed to solidify to then continue incubating at 37° for another 20 hours. This process was carried out as previously described in (Singh et al., 2022).

The analysis of the *D*- and *z*- values were performed by collecting the TDT disk from the ice bath and placing plain dough samples into stomacher bags with 10 ml of chilled, sterilized 0.1% peptone water inside. Samples were then stomached (Stomacher® 400 Circulator, Worthing, West Sussex, United Kingdom) for 1 min at 300 rpm, serially diluted onto the BHI agar and then inverted and incubated at 37°C for 4 hours. Samples

were then overlaid (~15 ml) with MacConkey agar, solidified, and then further incubated at 37°C for 20 hours. The number of STEC cells were counted using a Reichert Quebec Darkfield Digital Colony Counter-110 V (Buffalo, New York, USA) and represented as colony forming units (CFU/g).

Results:

At the end of the boiling process the internal temperature of bagels reached 107.7°C ±3.7°C as shown in **Figure 14**. The internal temperature of bagels during the 14 min of baking reached a maximum of 212.8°C ±0.28°C. At the end of an additional 15 min of ambient air cooling the mean internal temperature of plain bagels was 140.9 °C ±1.2°C (**Figure 15)**. The 2 min of boiling paired with 14 min of baking and 15 min of ambient air cooling resulted in reduction of 5.5 ±0.074 CFU/g of STEC population (**Figure 16**). A significant reduction of >4 log CFU/g in the STEC population was observed between 3.5 to 7 min into the baking. A lower detection limit of 0.4 log CFU/g was established in this study. As seen in **Figure 17,** the humidity ratio in the oven was 0.00549 ±0.00018 kg moisture/kg air at the beginning of baking and 0.45243 ±0.01923 kg moisture/kg air at the end of baking. **Figure 18** shows the bagel validation set up which includes boiling, baking and cooling.

As seen in **Figure 19**, the internal crumb temperature of the bagels showed no significant difference throughout the boiling and baking process. The crumb a_w varied from 0.932 ±0.006 to 0.950 ±0.004 while the crust a_w ranged from 0.834 ±0.03 to 0.933 ±0.007. The water activities of plain bagels crust were all similar except at 14.0 min of

baking. However, the water activity of post baked plain bagel was significantly different from all other readings. The water activity of plain bagels crumb at post proof, 1 and 2 min of boiling and 10.5 min of baking were significantly different from both the 14.0 and the 29.0 min crust samples. A significant increase in pH in the bagels occurred during the boiling and baking process. As seen in **Figure 20**, the pH ranged from 5.28 ±0.043 during pre-proofing to 5.83 ±0.023 at the end of 15 min of ambient air cooling. Also, the post proof sample was significantly different from every sample after the 1 min boil sample. The pre proof and 1 min boil samples were significantly different from every sample after 3.5 min of baking. Additionally, the 2 min boil sample was significantly different from the post proof sample as well as every sample after 3.5 min of baking.

During the *D*- and *z*- value study, the inoculated flour retained 7.2 ±0.10 log CFU/g STEC while the dough retained 6.9 ±0.17 log CFU/g. A linear regression plot was drawn by plotting the log population *vs* time, wherein three different temperatures were used in calculating the *D*-value for this STEC cocktail. In for the temperatures 56°C, 59°C and 62°C gave the respective *D*-values of 26.3 ±1.55 min, 9.0 ±0.27 min and 2.5 ±0.15 min (**Figure 21**). The log of these *D*-values were taken and plotted against temperature (°C) to get the *z*-value of 5.8 ±0.16°C (**Figure 22). Table 9** displays the calculated *D*- and *z*- values.

Discussion

Flour is a minimally processed raw agricultural product and requires thorough cooking before consumption (Crowe et al., 2017). Furthermore, it is a low water activity

ingredient that is typically not conducive to bacterial growth (Crowe et al., 2017). Despite this, pathogenic microorganisms such as STEC on wheat and its derivatives can withstand the drying process and persist in flour for weeks in a desiccated state (Beuchat et al. 2013; Burgess et al., 2016). The STEC, which is estimated to cause 265,000 infections in the United States annually, has been identified as one of the potential pathogens that can contaminate flour (Crowe et al., 2017).

The pH of plain bagels increased significantly from pre-proofed (5.28) to 7 min into the baking process, reaching a final value of 5.83. However, the pH remained unchanged from 7 min into the baking to 15 min after the ambient air cooling. Earlier studies also reported similar trends. In a validation study using plain muffins by Channaiah et al. (2017) the pH of the muffin batter increased from 6.61 ±0.12 to 7.49 ±0.04 by the end of 21 min of baking. Similarly, the pH of nut muffin batter increased from 6.50 ±0.21 to 7.37 ±0.05 at the end of 21 min of baking during validation of the nut muffins baking process (Channaiah et al., 2019a). The a_w of the crust portion of the plain bagel samples decreased significantly (0.933 to 0.885) by the end of the 29 min (baking + cooling). The decrease in a_w in plain muffins could be attributed to being directly exposed to oven heating while baking. However, the a_w of crumb portion of plain bagels remained unchanged (0.933 to 0.933), during the 29 min (baking + cooling) and the hardening and toughening of the crumb during baking could be the reason for this.

The plain bagel baking validation resulted in a reduction of 5.5 ±0.074 log CFU/g in STEC population by the end of the baking process, with a >5 log CFU/g reduction being achieved after 7.0 min of baking. Baking bagel baking at 450°F for 14 min plus 15 min of ambient air cooling resulted in >5 log CFU/g reduction in STEC population.

Earlier studies also reported similar findings. Singh & Channaiah (2022) were able to demonstrate >5 log CFU/g reduction in *Salmonella* population during traditional crust pepperoni pizza baking process when baked at 500°F (260°C) for 12 min. Likewise, baking plain muffins at 176.67°C (375°F) for 21 min resulted in >5 log CFU/g reduction in *Salmonella* population at the end of the baking (Channaiah et al., 2017). The lower limit of detection determined in this bagel study was 0.4 log CFU/g, though the mean log reduction never got this low.

The *D*- values of plain bagels in this study at 56, 59 and 62°C were 26.3 ±1.55, 9.0 ±0.27 and 2.5 ±0.15 respectively, and the *z*- value was 5.8 ±0.16°C. In a similar study by Suhalim et al. (2023) the investigators determined the *D*- and *z*- values of STEC, *Salmonella,* and *Enterococcus faecium* NRRL B-2354 in peanut butter, oatmeal, and chocolate chip cookie formulations at three moisture levels.

Suhalim et al. (2023) reported that at the lowest moisture and water activity level (8% moisture, a_w 0.421) of peanut butter cookies the *D*- values for STEC were 14.01, 3.56, 1.73 min at 65, 70, 75°C, while the *D*- values for *Salmonella* were 4.97, 2.59, 1.23 min, and for *E. faecium* were 15.50, 5.02, 2.02 min at 75, 80, and 85°C, respectively. The z-values for STEC, *Salmonella*, and *E. faecium,* were 11.02, 16.45 and 11.31°C, respectively. In comparison to the three cookie types, the *z*- value found in bagel baking was much lower, indicating less temperature is required to increase the kill rate when compared to these cookies. This could possibly be due to the lower a_w of the above three cookies when compared to plain bagels. Additionally, the increased sugar, protein and fat found in these cookies (in comparison to plain bagels) could also result in more shielding of STEC, resulting in increased heat resistance. In a study done by Singh & Channaiah

(2022) on STEC in pizza dough, the *D*- values were found to be 49.5, 15.3 and 2.8 min at the respective temperatures 55, 58 and 61°C. The *z*- value determined in this study was 4.8°C. The *z*-value found in this study was quite similar to that found in bagels, though the lower *z*-value could also be attributed to the varying protein and fat levels in the two products. Additionally, the heat resistances of STEC strains used in each study may vary in different matrices, resulting in various *z*- values.

Conclusion

This study validates and documents the first scientific evidence that baking a plain bagel at 450°F for 14 min serves as an effective kill-step by controlling STEC population by a >5 log CFU/g. The *D*- and *z*- values of STEC determined in this study will help optimize the baking process, thus achieving food safety. Furthermore, the *D*- and *z*- values of STEC determined in this study can be helpful in developing STEC predictive models for this matrix. It is important to note that the *D*- and *z*- values of STEC determined in this study are specific to the plain bagel recipe and baking parameters used. It is also worth noting that the *D*- and *z*- values will vary if any extrinsic or intrinsic properties are changed. Therefore, it is important not to extrapolate the results from this study. Individual validation studies specific to the product and pathogen are highly recommended if there is a change in the recipe or baking parameters.

APPENDIX: TABLES AND FIGURES -

Table 1: A list of baking validation research and associated heat resistant (*D*- and *z*-) characteristics.

Author	**Date Published**	**Bakery Product**	**Bacteria of Study**	**Time and Temp. to reach a 5-log reduction**	**D-Values**	**z-value**
Amanda Lathrop	April 2014	Peanut Butter Cookies	*Salmonella*	13 min at 177°C (350°F)	-	-
Lakshmikantha H. Channaiah	April 2016	Hamburger bun	*Salmonella*	6.0 min at 218.3°C (424.94° F)	28.64, 133.33, and 7.61 min at 55, 58, and 61°C	6.58° C
Lakshmikantha H. Channaiah	June 2017	Muffins	*Salmonella*	17 min at 190.6°C (375°F)	62.2, 40.1 and 16.5 min at 55, 58 and 61°C	10.4° C
Lakshmikantha H. Channaiah	December 2018	Donuts	*Salmonella*	2 min at 190.6°C (375°F)	8.6, 2.9, and 2.1 min at 55, 58, and 61°C	10.0° C
Lakshmikantha H. Channaiah	April 2019	Nut Muffins via inoculated flour	*Salmonella*	17 min at 190.6°C (375°F)	24.0, 4.0 and 0.6 min at 60, 65 and 70°C	6.1°C
Lakshmikantha H. Channaiah	April 2019	Nut muffins via inoculated walnuts	*Salmonella*	17 min at 190.6°C (375°F)	22.0, 3.6 and 1.7 min at 60, 65 and 70°C	9.0°C
Lakshmikantha H. Channaiah	September 2019	Whole Wheat Multigrain bread	*Salmonella*	15 minutes at 190.6°C (375°F)	59.6, 20.0, and 9.7 min at 50, 52, and 55°C	6.5°C

Minto Michael	March 2020	Plain Muffins	*Escherichia coli* 0121	17 min at 190.6°C (375°F)	42.0, 38.4 and 7.5 min at 60, 65 and 70°C	5.0°C
Lakshmikantha H. Channaiah	December 2020	Flour Tortilla	*Salmonella*	30, 45, and 60s at 221.1°C (430 °F)	22.2, 13.4, and 4.6 min at 55, 58, and 61°C	8.9°C
Phoebe Unger	January 2021	Brownie	*Salmonella*	40 min at 176.7°C (350°F)	53.4, 27.2 and 10.7 min at 64, 68 and 72°C	11.1° C
Phoebe Unger	January 2021	Brownie	*Listeria monocytogenes*	40 min at 176.7°C (350°F)	37.5, 16.9, 9.1 min at 64, 68 and 72°C	16.4° C
Lakshmikantha H. Channaiah	December 2021	Soft Cookies	*Salmonella*	11.5 min at 185°C (365°F)	62.3, 28.6 and 14.4 min. at 60, 65 and 70°C	15.8° C
Lakshmikantha H. Channaiah	December 2021	Hard Cookies	*Salmonella*	20.5 min at 185°C (365°F)	59.6, 28.1 and 11.9 min 60, 65 and 70 °C	14.5° C
Arshdeep Singh	October 2022	Crust Pizza	STEC *Escherichia coli*	12 min at 260°C (500°F)	49.5 ± 4.10 15.3 ± 0.68 and 2.8 ± 0.31 min at 55, 58 and 61°C	4.8 ± 0.16° C

*Note, all D value minutes and temperatures are listed in their respective orders

Table 2: *Salmonella* cultures used in plain bagel validation study.

Strain	ATCC® No.	Origin
Salmonella Typhimurium	14028	Tissue from pools of heart and liver from 4-week-old chickens
Salmonella Newport	6962	Food poisoning fatality
Salmonella Senftenberg	43845	N/A
Salmonella Tennessee	10722	N/A
Salmonella Agona	BAA-707	Plant
Salmonella Montevideo	BAA-710	Patient with salmonellosis associated with tomatoes (clinical specimen)
Salmonella Mbandaka	51958	N/A

Table 3: Plain bagel dough recipe used in baking validation study.

Ingredient	Weight (g)
Inoculated bread flour	250
Bread flour	450
Sugar	21
Salt	14
Dry yeast	5.6
Water	350

Table 4: Bagel dough recipe used in determining *D*- and *z*- values of *Salmonella*.

Ingredient	Weight (g)
Inoculated bread flour	140
Sugar	4.2
Salt	2.8
Water	70

Figure 1: Set up of inoculated plain bagel boiling process.

Figure 2: Set up of inoculated plain bagel with K-type thermocouple and MOLE data logger during baking validation study.

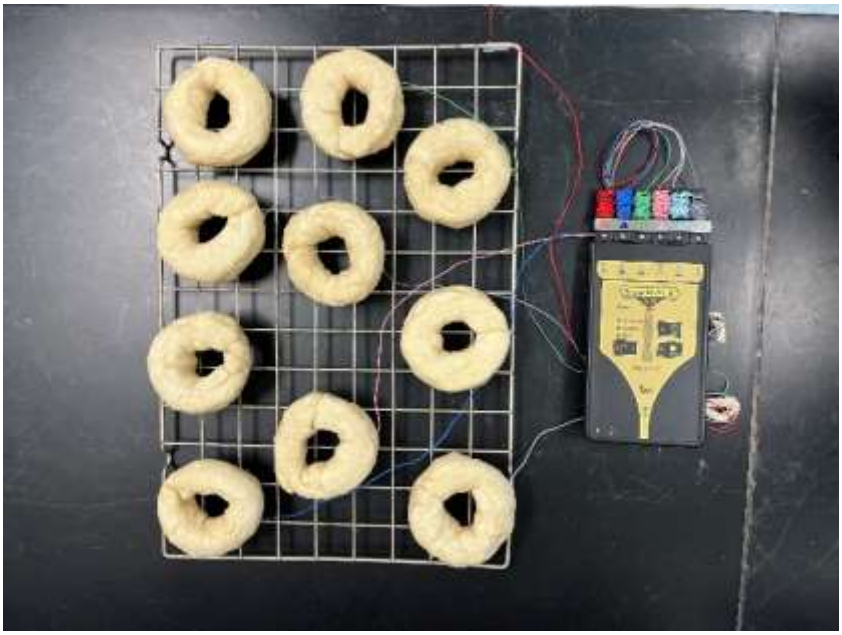
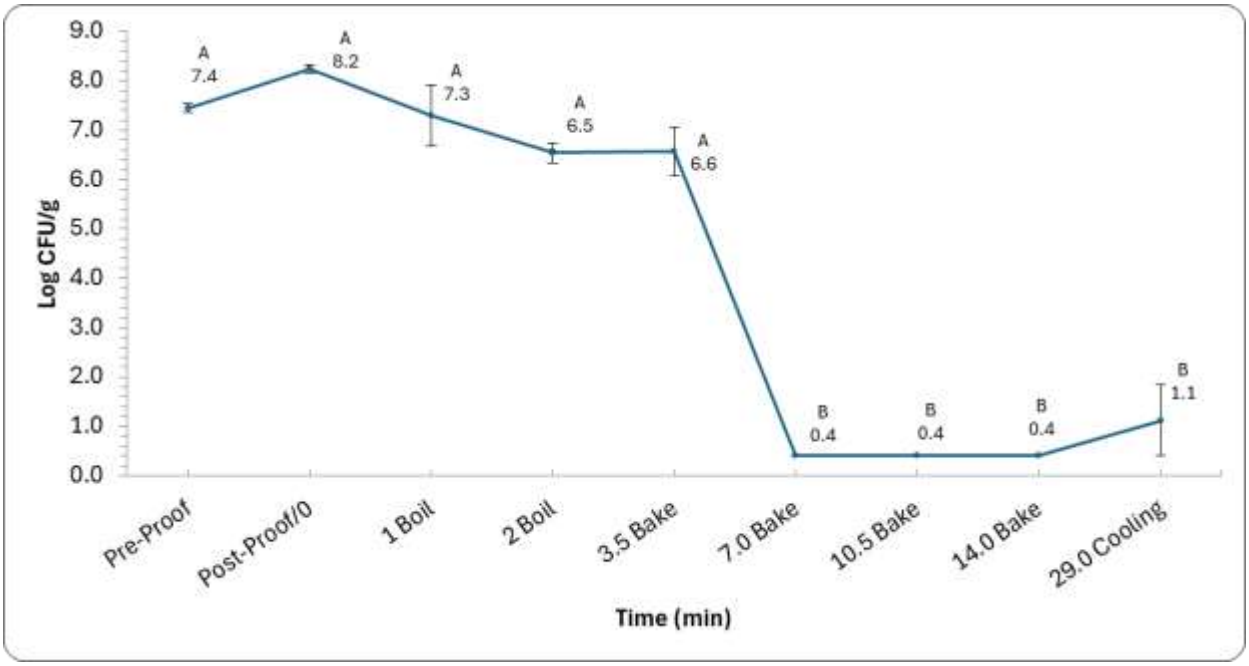


Figure 3: The mean $\log_{10}$ reduction (±SE, n=3) in *Salmonella* population during 14 min of baking (450°F) followed by 15 min of ambient air cooling when flour was used as the source of inoculum.



*Data points with different letters are significantly different ($p<0.05$)

*Detection limit is 0.4 log CFU/g

Figure 4: Mean internal temperature (±SE, n=3) of plain bagels during boiling.

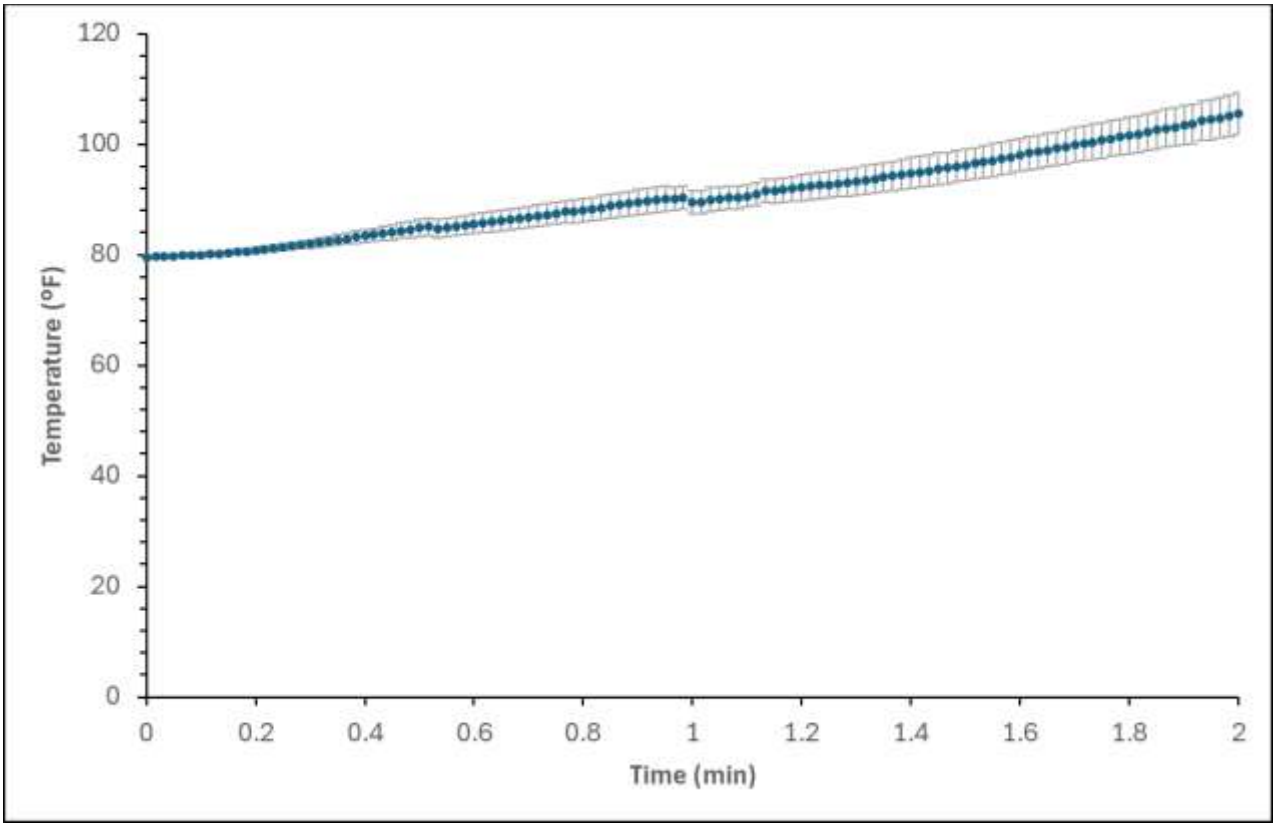


Figure 5: Mean internal temperature (±SE, n=3) of plain bagels inoculated with *Salmonella* during 14 minutes of baking at 450°F followed by 15 minutes of ambient air cooling.

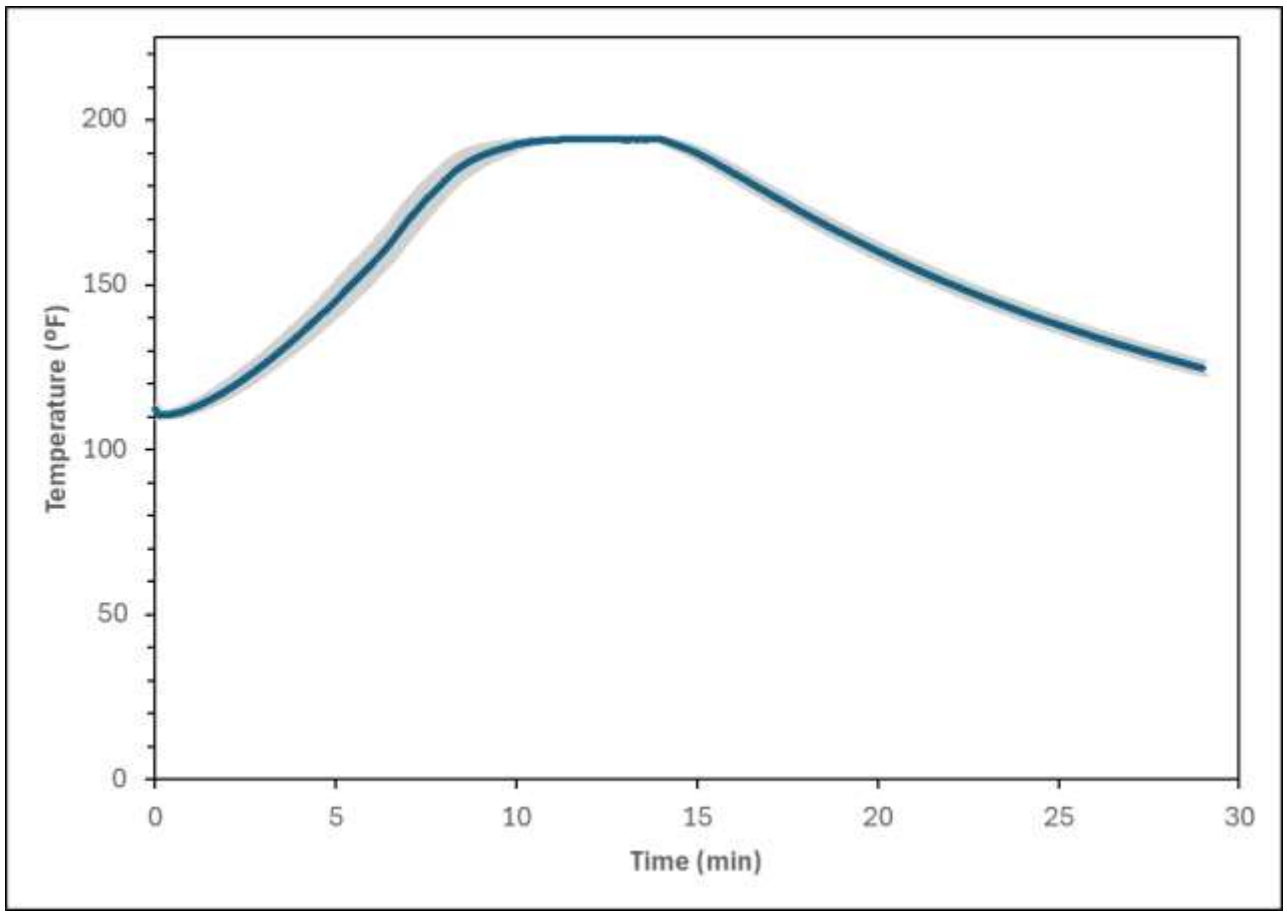


Figure 6: Mean humidity ratio (kg moisture/kg dry air) (±SE, n=3) of the oven during 14 min plain bagel baking at 450°F oven temperature.

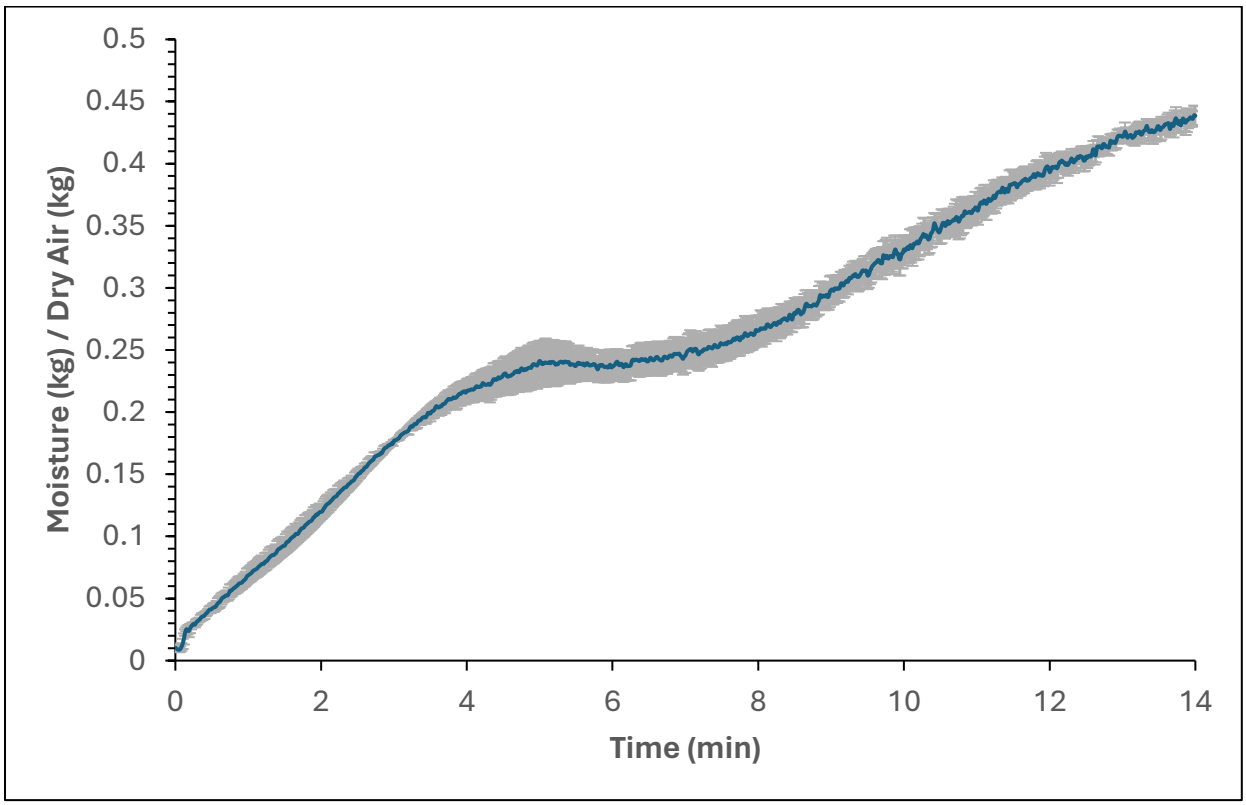
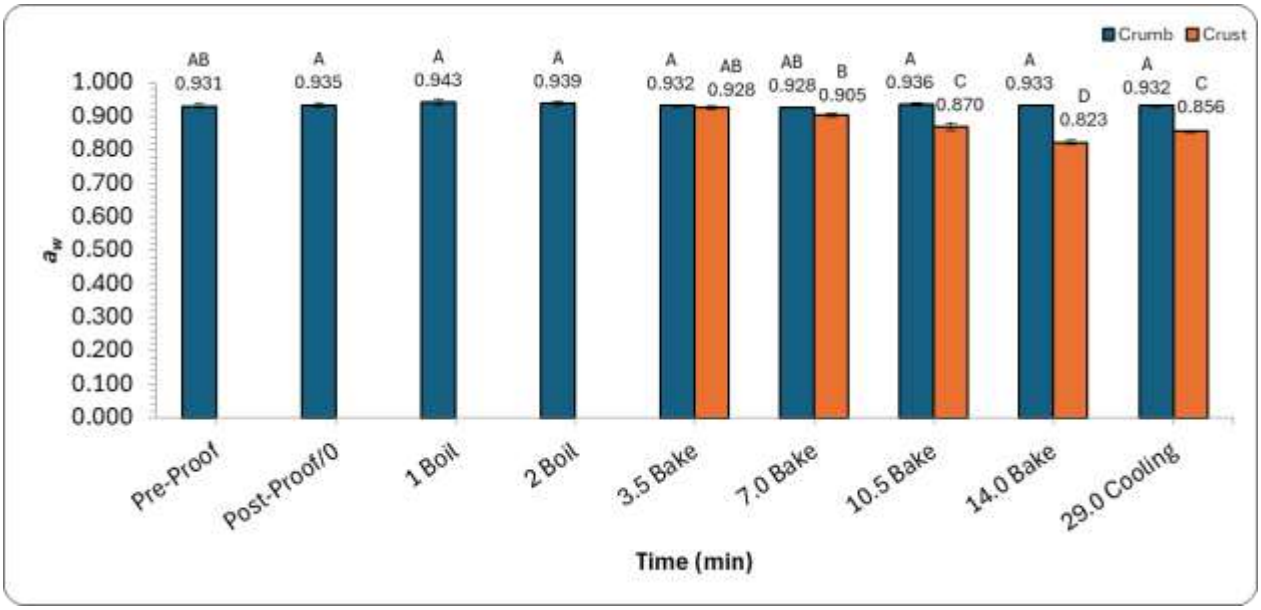


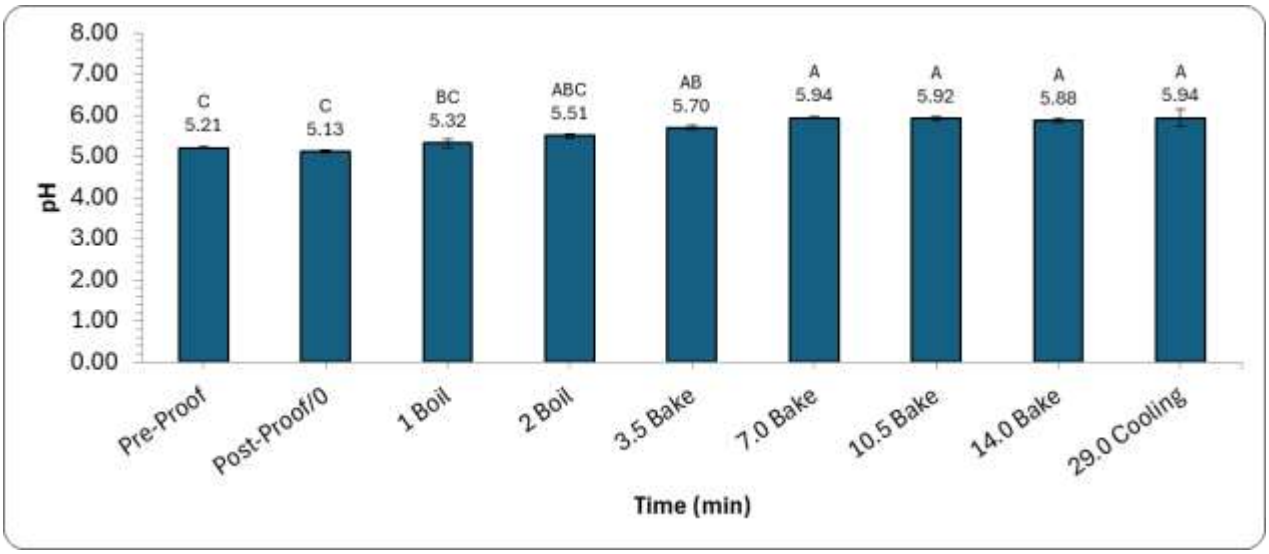
Figure 7: Post baked plain bagels.

Figure 8: The water activity (a_w) (±SE, n=3) of the bagels during 2 min of boiling, 14 min of baking and after 15 minutes of ambient air cooling.



*Data points with different letters are significantly different ($p<0.05$).

Figure 9: The pH (±SE, n=3) of the bagels during 2 min of boiling, 14 min of baking and after 15 minutes of ambient air cooling.



*Data points with different letters are significantly different ($p<0.05$).

Figure 10: Graph showing thermal inactivation of 7-serovar *Salmonella* populations (log CFU/g) *vs* time (min) (±SE, n=3) used for *D*- value calculation in bagels.

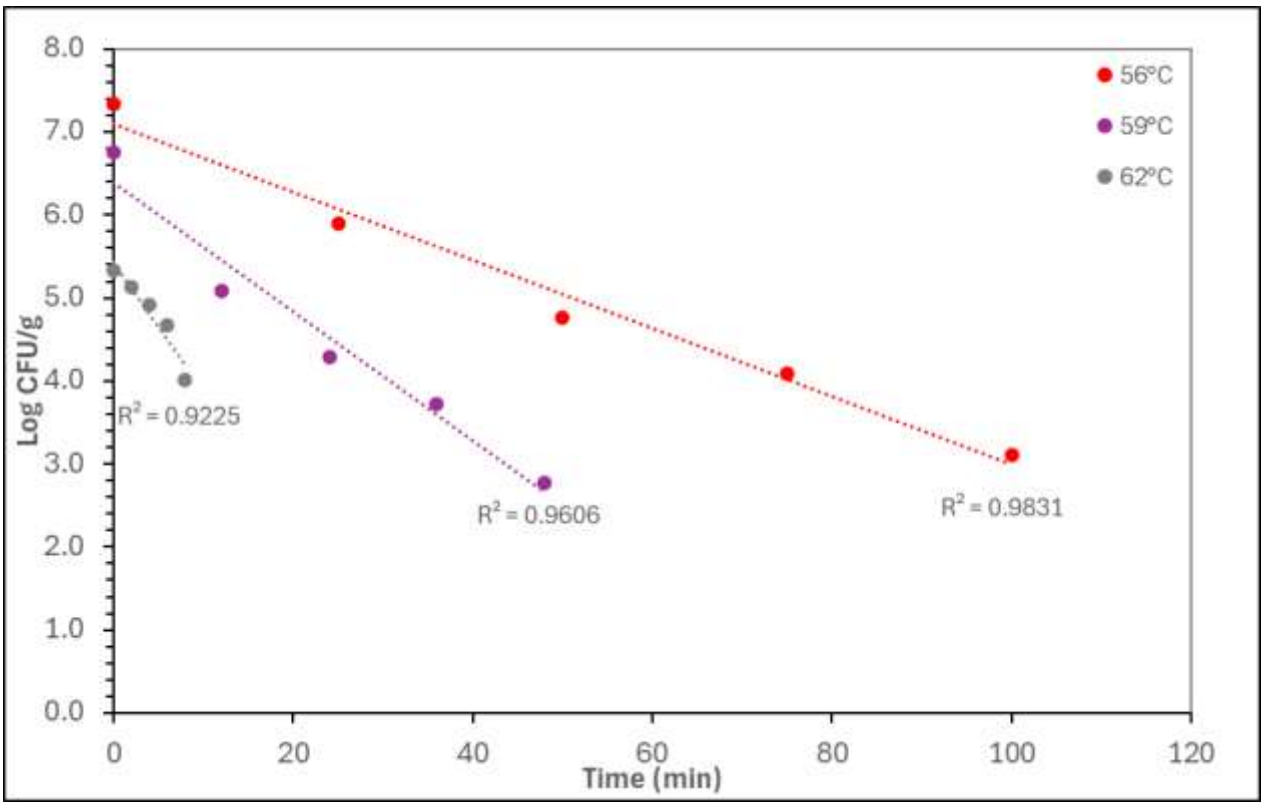


Figure 11: Graph showing log *D*-values [mean] versus temperature (°C) used for calculating *z*-values for the 7-serovar *Salmonella* cocktail.

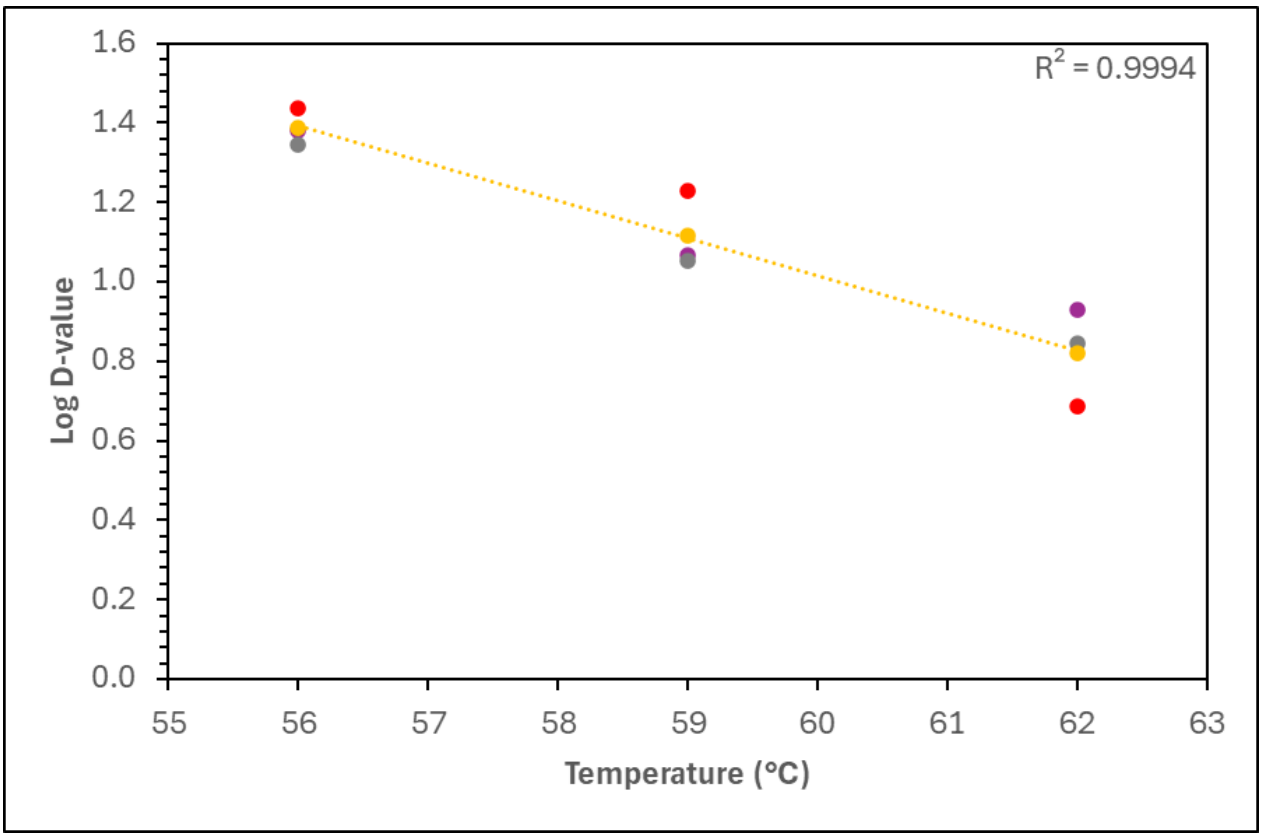


Table 5: Mean (±SE, n=3) *D*- (min) and *z*- (°C) values of *Salmonella*.

Items	*Salmonella*
56 °C	24.5±1.50
59 °C	13.3±1.85
62 °C	6.8±1.05
z- value	11.1±1.58

Table 6: List of STEC strains used in this study.

STEC	Strain	Origin
E. coli O157:H7	ATCC 43895	*Escherichia coli* strain (CDC EDL 933) was isolated from raw hamburger meat implicated in a hemorrhagic colitis outbreak.
E. coli O157:H7	ATCC 43894	*Escherichia coli* strain (CDC EDL 932) was isolated from patient's feces from outbreak of hemorrhagic colitis
E. coli O157:H7	C7927	Isolated from a patient from an outbreak linked to apple cider (CDC)
E. coli O157:H7	MF 1847	Source: (USDA-FSIS). Originally isolated from hamburger meat, Food Microbiology Laboratory, Howard University, USA
E. coli O26:H11	ATCC BAA-2196	Isolated from patient's stool in Michigan

Table 7: Recipe used for plain bagel validation study.

Ingredient	Weight (g)
Inoculated bread flour	250
Bread flour	450
Sugar	21
Salt	14
Dry yeast	5.6
Water	350

Table 8: Plain bagel recipe used for *D*- and *z*- value study.

Ingredient	Weight (g)
Inoculated bread flour	140
Sugar	4.2
Salt	2.8
Water	70

Figure 12: Plain bagel boiling set up.

Figure 13: Plain bagel baking set up with K-type thermocouples.

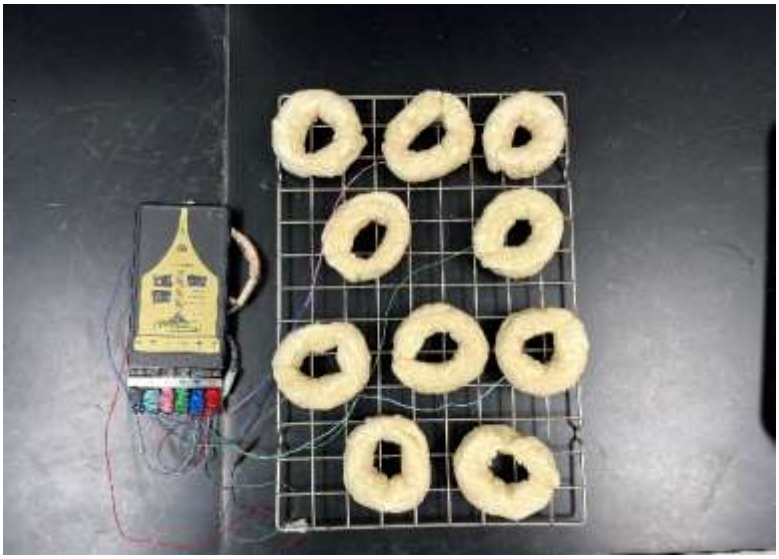


Figure 14: Mean internal temperature (±SE, n=3) of plain bagels during boiling.

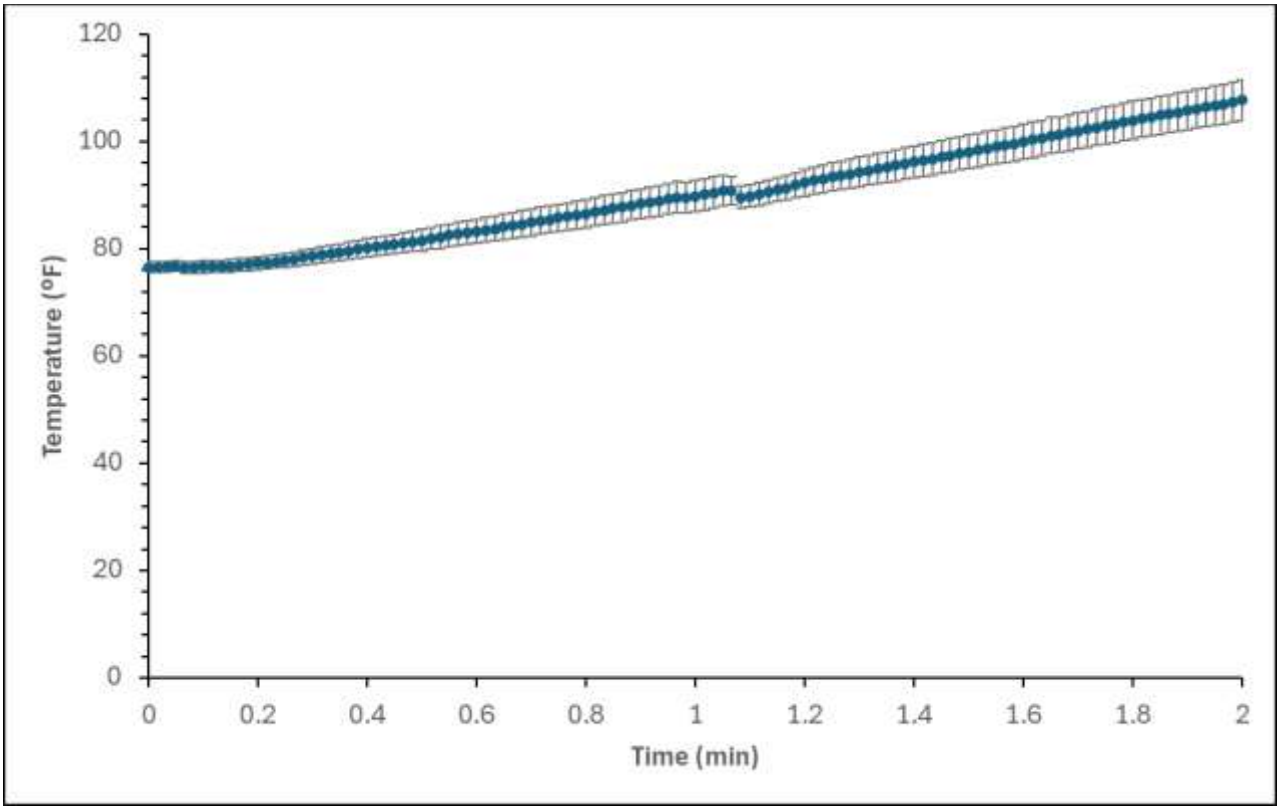


Figure 15: Mean internal temperature (±SE, n=3) of plain bagels inoculated with STEC during 14 minutes of baking at 450°F followed by 15 minutes of ambient air cooling.

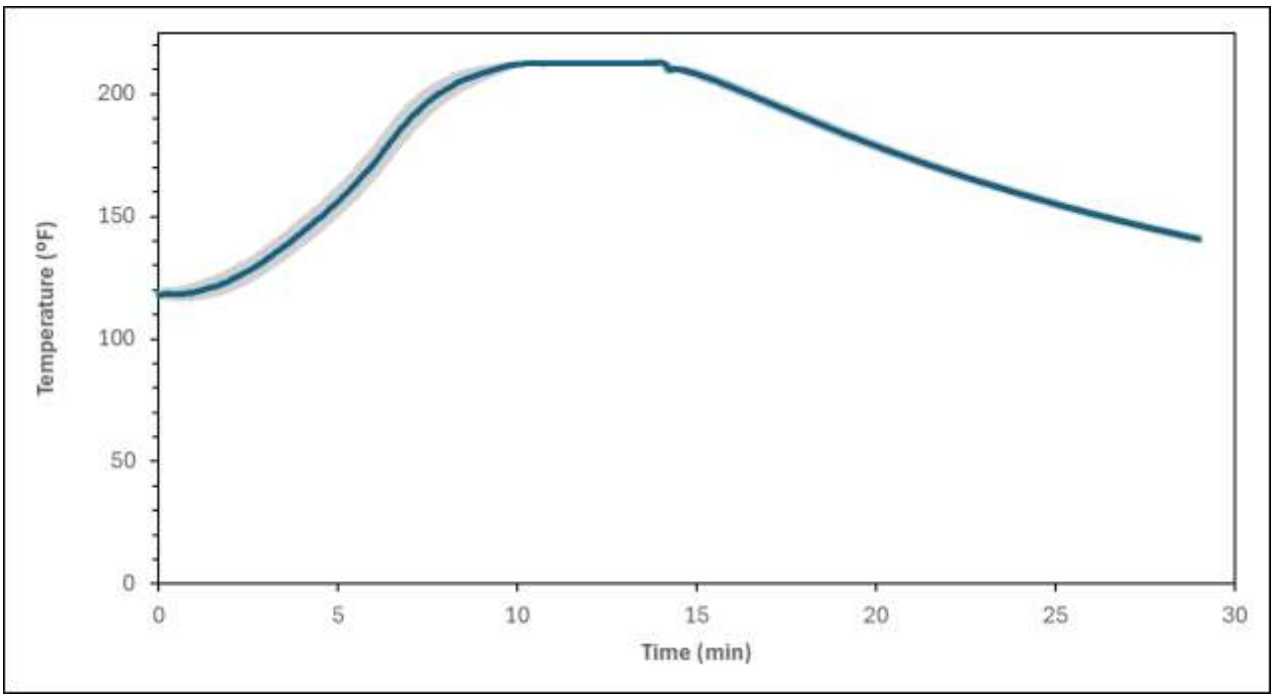
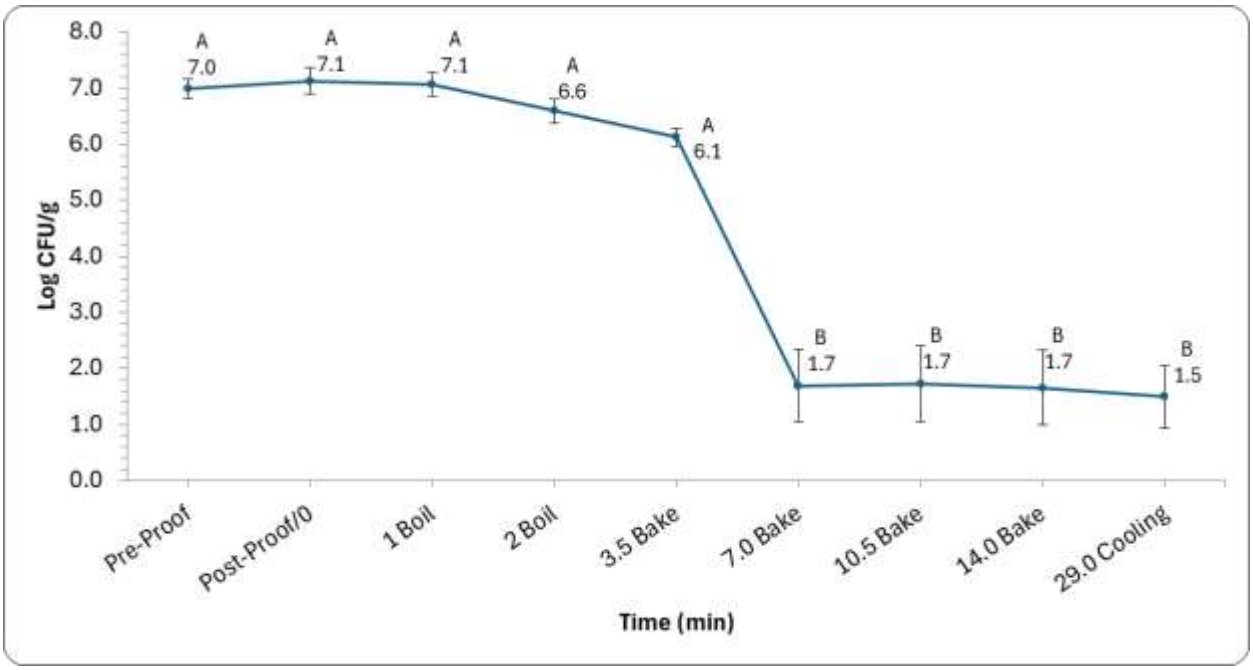


Figure 16: The mean $\log_{10}$ reduction (±SE, n=3) in STEC population during 14 min of baking (450°F) followed by 15 min of ambient air cooling when flour was used as the source of inoculum.



*Data points with different letters are significantly different ($p<0.05$)

*Detection limit is 0.4 log CFU/g

Figure 17: Mean humidity ratio (kg moisture/kg dry air) (±SE, n=3) of the oven during 14 min plain bagel baking at 450°F oven temperature.

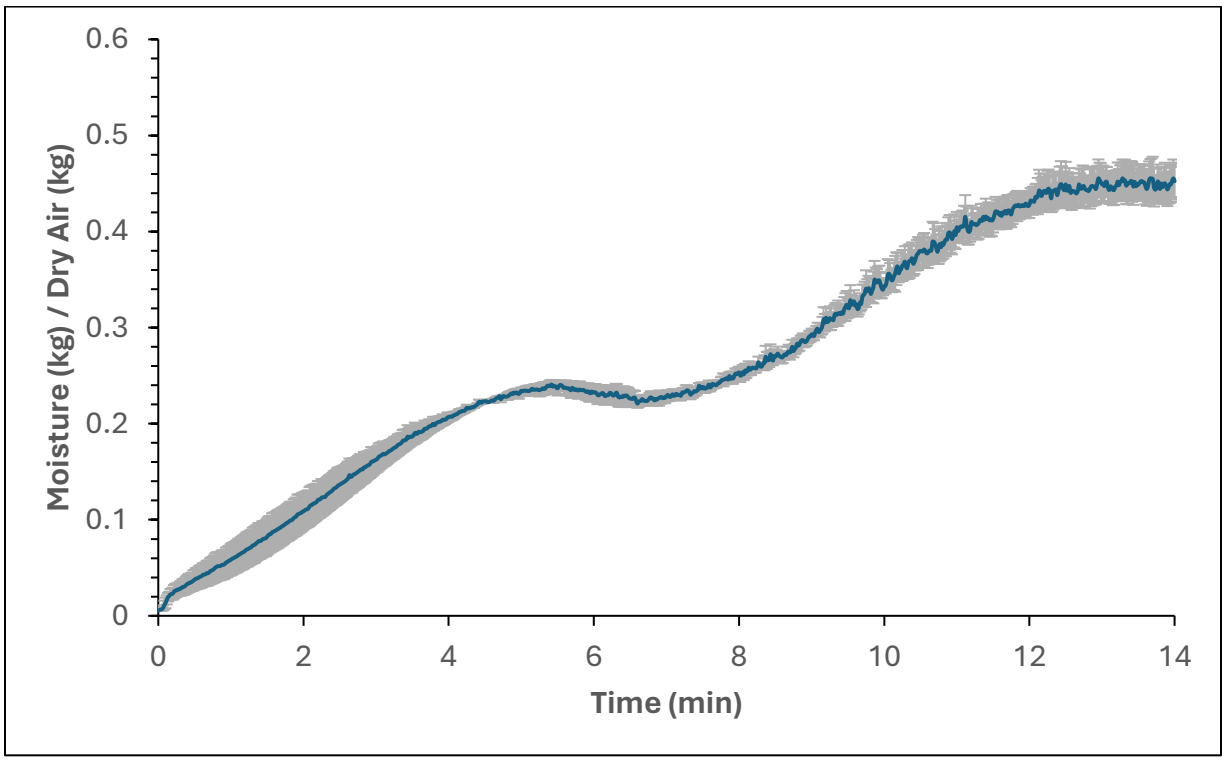
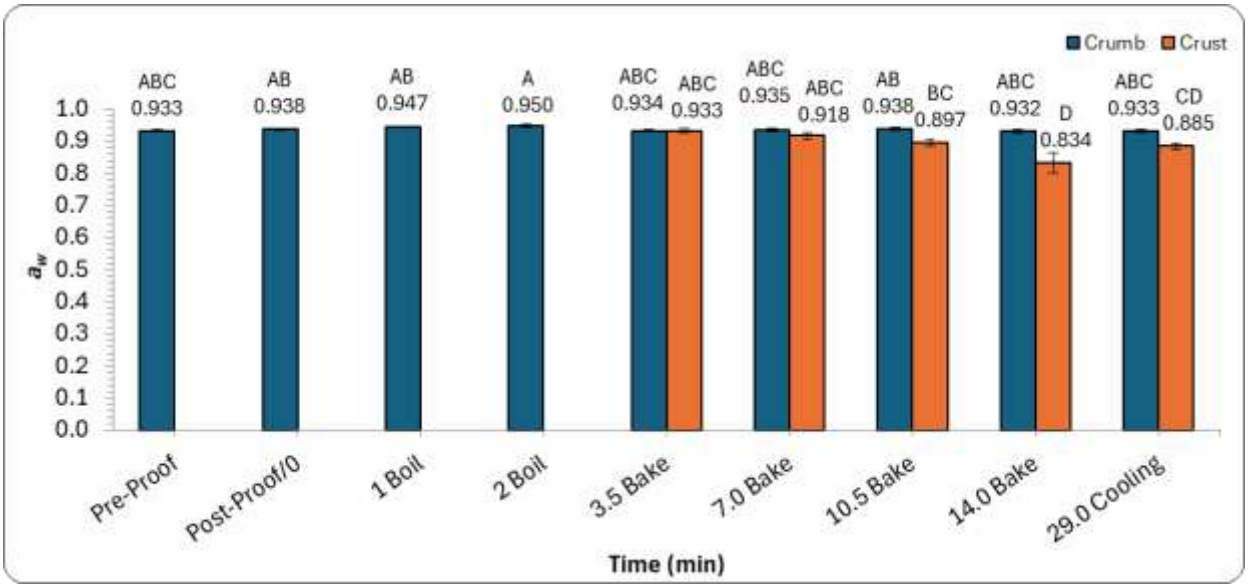


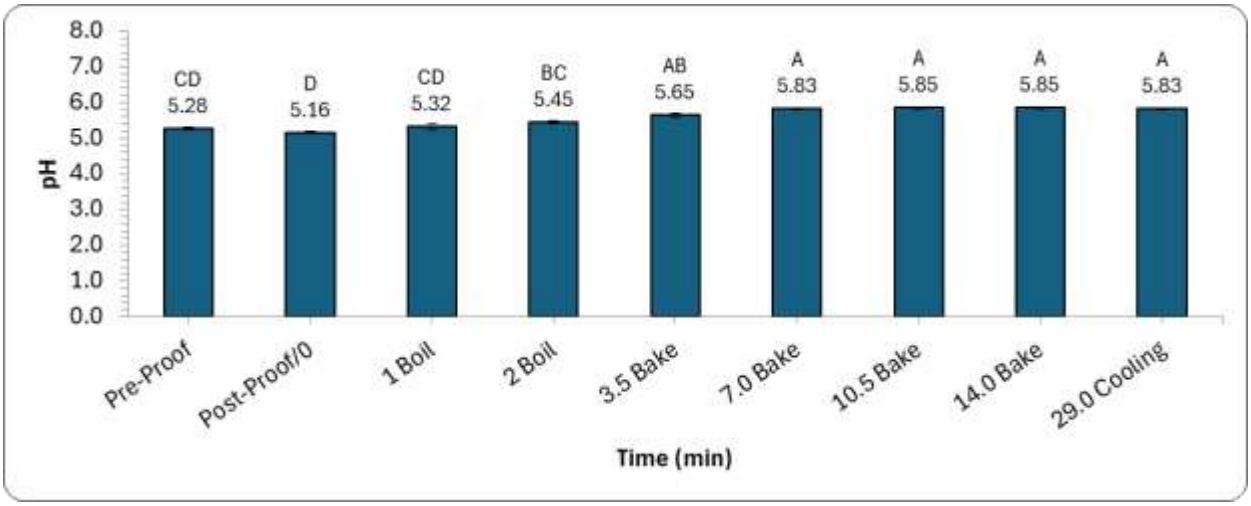
Figure 18: Plain bagels post baking with K-type thermocouples.

Figure 19: The water activity (a_w) (±SE, n=3) of the bagels during 2 min of boiling, 14 min of baking and after 15 minutes of ambient air cooling.



*Data points with different letters are significantly different ($p<0.05$).

Figure 20: The pH (±SE, n=3) of the bagels during 2 min of boiling, 14 min of baking and after 15 minutes of ambient air cooling.



*Data points with different letters are significantly different ($p<0.05$).

Figure 21: Graph showing thermal inactivation of 5-strain STEC populations (log CFU/g) *vs* time (min) (±SE, n=3) used for *D*- value calculation in bagels.

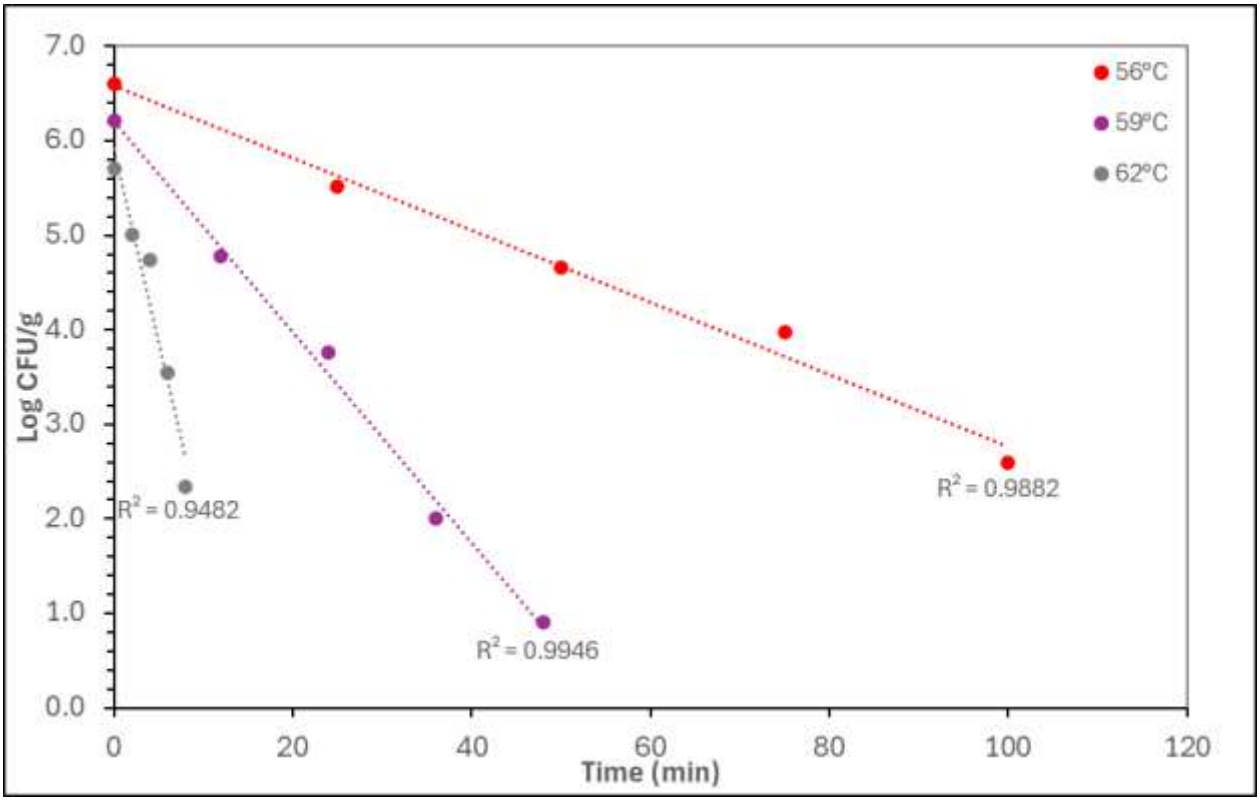


Figure 22: Graph showing log *D*-values [mean] versus temperature (°C) used for calculating *z*-values for the 5-strain STEC cocktail.

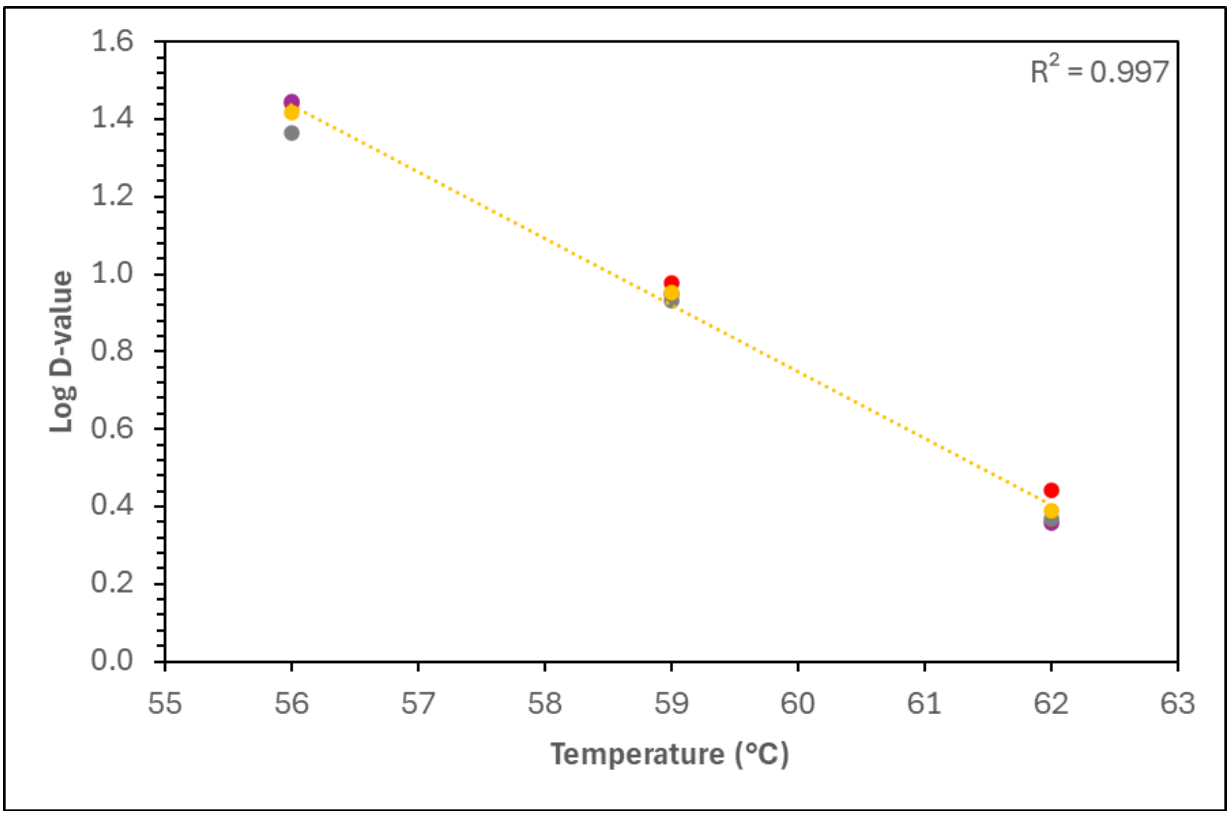


Table 9: Mean (±SE, n=3) *D*- (min) and *z*- (°C) values of STEC.

Items	STEC
56 °C	26.3±1.55
59 °C	9.0±0.27
62 °C	2.5±0.15
z- value	5.8±0.16

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