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Survival of Salmonella Strains in Ground Beef Containing Varying Fat Contents and Heated at Varying Calculated Lethalities

Christopher Jerome Williams
University of Tennessee - Knoxville

To the Graduate Council:

I am submitting herewith a thesis written by Christopher Jerome Williams entitled "Survival of Salmonella Strains in Ground Beef Containing Varying Fat Contents and Heated at Varying Calculated Lethalities." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Food Science and Technology.

John R Mount, Major Professor

We have read this thesis and recommend its acceptance:

P. Michael Davidson, F. Ann Draughon

Accepted for the Council:
Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

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Survival of Salmonella Strains in Ground Beef Containing Varying Fat Contents and Heated at Varying Calculated Lethalities

A Thesis Presented for the Master of Science Degree
The University of Tennessee, Knoxville

Christopher Jerome Williams
December 2009

Abstract

Americans consume 28 lbs of ground beef annually. Beef can become contaminated with *Salmonella* during the initial slaughter process. Whole cuts of meat are ground into other products and the contamination spreads. This increases the risk of food borne illness for many Americans that consume ground beef products. The purpose of this study is to determine if adequate microbial destruction of *Salmonella* populations in ground beef of different fat levels can be achieved at temperatures that are lower than government guidelines.

Ground beef was inoculated with a four-strain mixture of *Salmonella.* Three fat levels of ground beef were used (10, 17, and 25% fat). Samples of ground beef (10g) were heated in a water bath to target temperatures of 60, 65.5, and 71.1°C. The heated samples were removed from the water bath at predetermined time intervals and cooled in an ice bath. *Salmonella* was enumerated on plate count agar (PCA). Serotype survival was also analyzed. The heat treatments significantly decreased bacteria populations ($p < 0.05$) and the 60, 65.6, and 71.1 °C heat treatments were significantly different from each other. The results indicate that fat level had no significant effect on bacterial survival ($p > 0.05$). Also, *S.* Senftenburg was found most often during longer exposure to heat treatment. Overall, the results indicate that combinations of heating at 60, 65.6 °C can achieve similar bacterial destruction as heating at 71.1 °C and should be considered by manufactures that use ground beef.

Table of Contents

List of Tables Page

List of Figures

Page

Figure:

List of Figures

Page

Figure:

Part I

Introduction

According to the American Meat Institute, 76% of all beef eaten at restaurants in the United States in the form are hamburgers (AMI 2005). Hamburgers and ground beef represent meals that can be consumed quickly. USDA recommends that ground beef should reach 160 °F internally and be cooked until brown with clear juices (FSIS 2002b). The Economic Research Service of the USDA reports that consumers prepare medium rare, rare, and pink hamburgers 16% of the time, increasing the chance of food borne illness. This fact along and instances of premature browning of ground beef below 160 °F increase the danger of *Salmonella* infections in ground beef (Suman and others 2004).

Ground beef becomes contaminated with bacteria when whole cuts of contaminated meat trimmings are mixed together. Whole cuts are contaminated during the slaughter process. Steam treatment of beef carcasses can reduce bacteria but does not eliminate vegetative bacteria (Huffman 2002).

Studies of the heat treatment effects on *Salmonella* have been conducted in eggs and meats such as chicken, pork, turkey, etc (Shuman and others 1997; Murphy and others 2002). Other heat reduction studies have been done with *E. coli* and ground beef (Juneja and others 1997). Fewer studies that address *Salmonella* reduction on whole cuts of beef and in ground beef have been conducted (Goodfellow and others 1978; Orta-Ramirez and others 2005).

Objective

The objective of this study was to compare microbial destruction of *Salmonella* in ground beef of different fat levels at heat treatments below the government specifications. The microbial destruction was determined immediately after the ground beef samples were heated in a water bath at target temperatures of 60, 65.6, and 71.1°C. Serotype analysis was performed on samples treated at 65.6 °C and 71.1 °C.

Part II

Literature Review

Salmonella

Salmonella are gram negative bacteria of the genus Enterobacteriaceae. *Salmonella* bacteria are non-sporeforming, facultative anaerobes that have a rod appearance under magnification. Five subgenera of more than 2,200 known serovars exist (Jay 1996). Subgenera I contains pathogenic forms founds in warm blooded animals. Subgenera's II and III contain serovars that can be found in cold blooded animals and subgenera's IV, V contain *Salmonella* that are common to the environment but rarely cause illness in humans. *Salmonella* species can be named by the location of discovery or disease associations.

Salmonella bacteria are considered to be ubiquitous in our environment and can survive for years under non-optimal conditions. S*almonella* contaminates food, people, and animals. Humans, mammals, reptiles, arthropods, fish, and amphibians have been identified as carriers of *Salmonella* bacteria (Jay 1996).

Animals are exposed to *Salmonella* in water, feed, dirt, feces, and insects and can become infected or non-infected carriers of the organism (Jay 1996). Humans ingest contaminated food and water and become infected with *Salmonella*. The activity of humans and other animals in contaminated areas spreads the organism to other animals and areas.

Salmonella has been identified in many foods and surface contamination of meat and poultry is common (Doyle and others 2000). *Salmonella* also contaminates the interior and exterior of eggs; raw milk; and animal feeds before and after processing. Salmonella Enteritidis, Salmonella Newport, and Salmonella Typhimurium are the most

commonly seen serotypes in the United States (CDC 2002a, 2002b). Due to the common presence of *Salmonella* in most meat species, USDA and FSIS regard *Salmonella* as an indicator for the presence of other pathogenic bacteria such as Listeria species and *E. coli* species (Aberle and others 2001). Processing plants use bacterial reduction and control strategies for *Salmonell*a to reduce other food pathogens.

Salmonellosis

The first reported outbreak of salmonellosis occurred in 57 people that ate beef during the late 1800's (Jay 1996). The Center for Disease Control estimates 40,000 salmonellosis cases per year and 500 deaths per year (CDC 2002a). The largest US outbreak involved more than 200,000 people in 41 states who consumed ice cream transported in tankers previously carrying liquid eggs (Jay 1996). Literature reports of the infectious dose for salmonellosis range from log 5 –log 7 CFU/g however, instances of the illness have occurred with lower levels of *Salmonella* (Jay 1996; Doyle and others 2000).

Salmonella infection occurs in humans, cattle, poultry, and swine. The duration of salmonellosis in humans is 2-3 days. Symptoms include nausea, muscle ache, fever, and gastroenteritis and occur within 12-14 hours (CDC 2002a). According to the CDC, multi-drug resistance of *Salmonella* serotypes has increased. Salmonella Newport and Salmonella Typhimurium DT104 present more concerns for people that become infected with salmonellosis as these serotypes are more common and have been identified as drug resistant to 9 antimicrobial drugs and 5 antimicrobial drugs respectively (CDC 2002a).

The organism is ingested through contaminated food, water, and remains in the intestines. Pathogenic *Salmonella* attack the lumen of the small intestine and multiply in number. Bacterial toxins produced by *Salmonella* cause fluid accumulation, increases in intestinal cell permeability, and intestinal mucosa cell destruction (Doyle and others 2000). Illness can result in further complications of septicemia and death. The death rate of salmonellosis illness is 4.1% but varies with causative species.

Salmonella in Ground Beef

Salmonella in beef is a cause for concern to the food industry (Juneja and others 2000; Murphy and others 2004). Food Safety and Inspection Service testing of HACCP-regulated processing plants during 1998-1999 indicated the presence of *Salmonella* isolates in ground beef (Table 1) (FSIS 2002c). Improper handling, cooking, and storage practices by consumers; premature browning of ground beef during cooking; and indications of the heat resistance of *Salmonella* attached to muscle tissue contribute to the necessary concern for this pathogen in ground beef (FSIS 2002a, 2002b, 2002c; Doyle and others 2000).

Feces, soil, and water can attach to the carcass hide and process equipment during slaughter and can contaminate other carcasses. *Salmonella* that are present in the rumen of healthy and infected cattle can contaminate the carcass if the rumen is punctured (Jay 1996). Beef carcasses are washed to remove soil and bacteria; however some pathogenic bacteria remain on the carcass. The cooling and freezing process of beef carcasses after slaughter reduces initial microbial loads but, *Salmonella* can continue to survive at

temperatures below freezing. Also, further thawing by the consumer may aid bacterial growth by releasing free amino acids, vitamins, and surface moisture (Jay 1996)

Heat Resistance of Bacteria

As cooking time and temperature increase, levels of microbial destruction increase. The rate of temperature increase in foods also affects thermal inactivation levels. Palumbo and others (1996) observed that increases in the rate of temperature change resulted in decreased time to reach target temperature and increased destruction of *Salmonella* in egg whites. Meat species, cut selection, additional ingredients, water holding capacities, and heating method can affect the heating rates and the final internal temperature.

D-values and z-values are used in the food industry to determine the effectiveness of the heat inactivation process. D-value is defined as the time required to reduce microbial counts at a constant temperature by 90% (Verramuthu and others 1998; Murphy and others 2004; Jay 1996). Z-value is the temperature change needed to achieve a 90% decrease in D-value (Verramuthu and others 1998; Pflug 1997; Jay 1996). D-values and Z-values are product specific and are difficult to apply between different products and different processes (Shaw and others 2000; Murphy and others 2001). Heat resistance occurs when bacteria under similar heating conditions experience differences in D and z-values. S. Senftenberg is associated with heat resistance in foods and is commonly used in heat resistance, thermal death studies (Chantarapanont and others 2000; Doyle and others 2000; Kumar and others 2003).

Many factors contribute to bacterial heat resistance. These factors originate from either the food or the bacteria. Carbohydrates, fats, and water affect microbial heat resistance (Orta-Ramirez and others 2005; Smith and others 2001). Fat can serve as insulation for microorganism from heat (Ahmed and others 1995). Fat also prevents moisture loss in food systems. As a result, it is more difficult to destroy bacteria in foods of higher fat content. Line and others (1991), Fain Jr. and others (1991), and Smith and others (2001) observed that increases in fat level of meat increased D-values, bacterial counts after heat treatment. Larger D –values were also observed between meat products of higher fat levels when compared to D- values of meat products containing lower fat levels (Juneja and others 2000, 2001; Murphy and others 2004). Significantly different *Salmonella* D-values were seen in meat of different fat levels (Juneja and others 2000, 2001). Lastly, less microbial destruction was also observed in ground beef jerky and pepperoni of higher fat content when compared to ground beef jerky and pepperoni with lower fat content (Faith and others 1998a, 1998b).

Changes in the moisture levels of foods can also affect bacterial heat resistance. Shuman and others (1996) found that reducing solids and adding moisture in eggs resulted in a decrease in heat resistance of *Salmonella* species. Also, *E. coli* were more resistant to heat treatment when inoculated into ground beef that contained less moisture that other samples of ground beef (Shuman and others 1996).

Cell age contributes to heat resistance. Bacterial cells exhibit initial, logarithmic, stationary, and lag growth phases. Heat resistance is highest during the stationary growth phase. It is reported that S. Senftenburg 775W, a strain that is associated with heat

resistance, is more resistant at the stationary phase of growth (Jay 1996; Doyle and others 2000). Cells in the initial lag phase may also exhibit higher levels of heat resistance

Larger populations of bacteria exhibit more resistance to heat (Jay 1996). Energy absorbing, protective proteins have been identified in large populations of bacteria. Also, larger bacterial populations can produce more heat-resistant cells.

Sugars can affect heat resistance by affecting the water activity of the food. The amount of water activity change depends on the type of sugar and the sugar concentration. Jay (1996) reports the decreasing heat resistance of S. Senftenburg 775W in like concentrations of sucrose, glucose, sorbitol, and fructose respectively.

Salts can similarly affect bacterial heat resistance (Jay 1996; Doyle and other 2000). Salts can either increase or decrease the water activity of the food, thereby changing the bacterial resistance level. As with sugars, the effects on water activity depend on the type of salt and the salt concentration.

Bacteria are more resistant to heat at pH 7.0 or higher (Doyle and others 2000). Both increases and decreases in pH increase bacterial heat sensitivity. The use of organic acids has greater effects on the heat sensitivity of bacteria.

Beef Patty, Hamburger Industry

Ground beef consists of fresh or frozen beef with or without added seasonings, with no more than 25% beef cheek meat, and without additional beef fat. Hamburger consists of fresh or frozen beef with or without added seasonings and beef fat. Like ground beef, hamburger may not contain more than 25% beef cheek meat. Neither ground beef nor hamburger may contain more than 30% fat by weight and neither may

contain additional water, phosphates, binders or extenders (US Government Print Office 2007d).

Beef patties consist of fresh or frozen beef and may contain additional beef fat or seasonings. Beef patties may also contain binders, extenders, mechanically separated meat, and or partially defatted beef tissue that has been added with water at levels that will maintain the original characteristics of a beef patty (US Government Print Office 2007d)

Hamburgers and beef patties are comminuted products reduced in particle size by processes of chopping, flaking, grinding, or mincing (US Government Print Office 2007c). Comminuting is useful for spreading ingredients and increasing the tenderness of lesser-quality meat cuts (Romans and others 2001). However, ground products are higher in pathogens in comparison to whole cuts of meat (Murphy and others 2002; Romans and others 2001). Higher instances of pathogens in ground and comminuted products create more opportunities for food borne illness in the US.

Commercial meat patty manufacturers utilize batter processes. Batters represent all finely subdivided, viscous masses that exhibit emulsion characteristics (Romans and others 2001). Water, fat, salt, and cuts of muscle are sheared together at high speeds. In a two step process, meat proteins swell to form a meat matrix. Meat proteins and fat within the meat matrix create an emulsion (Romans and others 2001; Rankin 2000).

Consumer Trends in the Ground Beef Industry

Beef is the most popular red meat source among Americans. There are various factors that affect beef consumption levels in the US. Government and private agencies

have linked beef consumption to factors such as price, demographics, ethnicity, race, and age (USDA 2005; AMI 2005). Americans consume 67 pounds per year of beef at retail weight. At boneless weight, Americans consume 3 ounces per day of beef (USDA 2005). Beef consumption is highest in rural areas of the Midwest, in males age 20- 39 years of age, in Black households, and in low income families.

The categories of beef consumed are determined by the same factors that determine overall beef consumption. Ground beef is the most available protein source to Americans and is the largest category consumed by Americans in rural areas, among males age 12-19 years old, in lower income households, and in Black households (USDA 2005). Ground beef constitutes 42% of the total beef consumption is the US at a level of 28 pounds per person annually (AMI 2005). In restaurants, ground beef is 75% of all beef served. Households purchase ground beef at a rate of 5.6 pounds per month, which constitutes 65% of total beef consumption in the home.

A large portion of ground beef consumption is derived from the hamburger industry. Hamburgers are the largest portion of the US meat industry (Romans and others 2001). In the US, hamburgers are currently 76% of beef consumed outside of the home. Common serving sizes include 1/6 of a pound, 1/4 of a pound, and 1/3 of a pound.

According to the Market Research Corporation of America (MRCA), in 1996 consumers ate rare or medium-rare hamburgers 15% and 20% respectively (Ralston and others 2000). However, consumers who have previously contracted a food borne illness are less likely to eat hamburgers that are rare or medium rare. Current trends suggest that consumers prepare ground beef with a “cook until brown” approach. 160 °F for 1 second

should be sufficient to destroy pathogenic bacteria (FSIS 2002 b). However, ground beef can brown at temperatures below 160 °F. According to a FSIS study, 20.6% and 7.5% of ground beef prepared by US consumers became visually brown at temperatures of 150 °F and 135 °F respectively (FSIS 2002a). These findings suggest that government regulation regarding processing and cooking is needed to ensure the safety of ground beef in the US.

Beef Slaughter Process

Handling practices contribute to the overall safety and quality of raw meat and products containing raw meat. The handling steps at the slaughtering facility or during consumer preparation contribute to the total microbial load. Resulting microbial loads may be elevated to levels that significantly negate the microbial reduction that occurs during the preservation steps of freezing, cooking, chilling, and refrigeration. Pathogenic bacteria of concern in beef carcass decontamination are *E. coli* O157:H7, *Salmonella*, *Listeria, Campylobacter,* C. Botulinum, C. Perfringens, Stapylococcus Aureus, Aeromonuas Hydrophila, and Bacillus Cereus (Huffman 2002).

Most bacterial contamination of whole meat occurs as natural barriers and the immune system are destroyed during the slaughter process (Romans and others 2001). During slaughter, meat carcasses are trimmed, washed with a water-organic acid or water-chlorine mixture; and treated with steam. These steps facilitate the removal and destruction of pathogenic and spoilage bacteria present on the hide, skin, and vectors such as the visceral cavity, workers, and machinery (Jay 1996; Delmore and others 2000; Gill 2001, 2003). Organic acid and chlorination washes have demonstrated log 2 CFU/cm² reduction of surface contamination. However, beef carcasses can still contain

bacterial counts log 7 CFU/cm² and final counts are dependent on the length storage time before processing (Kenney and others 1994).

After slaughter, the beef carcass is cooled. The thickest portion of the carcass must reach a temperature below 5 °C (Bolton 2001; Hooker and Murano 2001). Carcass fat content, carcass size, and number of carcasses can affect the rate of cooling. Cooled carcasses are stored at 0-3 °C until cut. Carcasses are cut and vacuum packaged at temperatures above 5 °C which increases the possibility of microbial growth. Despite government regulations and sanitation procedures of slaughter facilities, Zhao and others (2002) found pathogenic *Salmonella* in retail sold ground beef.

Cooking and Heating Procedures

The most used meat preservation step is thermal processing. Thermal processing includes product heating, holding, and cooling steps. Cooking occurs at either moderate (58-75 °C) or high temperatures (100 °C+) (Aberle and others 2001). During the heating process, proteins denature and coagulate as water and fat migrate throughout the meat. Gelation, water entrapment, and fat entrapment can occur in commuted meat products such as meat patties, sausages and hot dogs (Aberle and others 2001).

Blanching represents cooking with mild heat. Steam, air, and water are used to heat meat at temperatures below 65 °C (Jay 1996; Aberle and others 2001). Blanching reduces surface bacteria and deactivates spoilage enzymes.

Pasteurization destroys pathogens and reduces spoilage bacteria. Thermophylic and thermoduric bacteria can survive pasteurization (Jay 1996; Aberle and others 2001).

Meat processing often utilizes a pasteurization step to simultaneously cook products and maintain sensory attributes.

Commercial sterilization is the most used cooking method (Aberle and others 2001). Commercial sterilization destroys all viable pathogens, yeasts, and molds capable of growth at non-refrigerated temperatures. The canning, aseptic packaging industry utilizes commercial sterilization processes to produce shelf stable products.

Regulation of Salmonella in Ground Beef

The Food Safety and Inspection Service (FSIS) is the branch of the United States Department of Agriculture (USDA) that ensures safety; wholesomeness; and labeling of meat, poultry, and egg products (FSIS 2006). FSIS receives responsibility from the Federal Meat and Inspection Act. In 1999, FSIS created Pathogen Reduction programs that established *Salmonella* and *E. coli* performance standards for slaughter facilities and raw ground products plants, control rules for preventative systems, and sanitation standards. When processing plants are operating within the *Salmonella* performance standard (Table 2), there is an 80% probability that the establishments will pass inspection (US Government Print Office 2007a, e).

Salmonella is prevalent in the environment and is used to evaluate the effectiveness of sanitation programs in FSIS inspected facilities. *Salmonella* and other pathogens are often found in cull/ market dairy cattle (Galland and others 2001; Scanga and others 2000). *Salmonella* was found in 23.1% of market dairy cattle samples processed for ground beef, therefore the US ground beef supply presents health risks to

consumers (Troutt and others 2001). Effectively controlling *Salmonella* reduces other food borne pathogens (FSIS 2002).

Salmonella standards use a national average baseline and are product specific. Cow/ bull carcasses, steer/ heifers, market hogs, broilers, ground beef, ground chicken, and ground turkey are inspected under FSIS Pathogen Reduction/ HACCP (USDA 2002). To date, FSIS provides inspection services at a cost of $2.20 per taxpayer (Romans and others 2001).

Heating Process Regulation

FSIS regulates the heat process of uncured meat patties through § 9 CFR 318.23, which defines meat patty standards and minimum heating requirements for official establishments (Table 4). Comminuted patties are partially-cooked, char-marked or fully-cooked (US Government Print Office 2007c). All heating processes must be able to prevent the multiplication of C. Botulinum with no more than 1 log CFU/g of C. Perfringens. All heating processes must be approved by FSIS and alterations to approved processes must be approved with scientific evidence. Lastly, companies will document and investigate all process deviations, properly handle questionable products, and properly label patties that are not fully-cooked (US Government Print Office 2007c).

The Department of Health and Human Services (HHS)/ Food and Drug Administration (FDA) enforces the United States Food Code for food establishments (FDA 2006). Chapter 3 of the US Food Code establishes heating/cooking regulations for the destruction of organisms of a public health concern in raw animal foods (Table 3). All regulated raw animal foods such as eggs, fish, meat, poultry, and foods containing these

items shall be cooked to heat all parts of the food to a time, temperature that complies with the methods of 3-401.11 of the US Food Code (FDA 2006). FDA and FSIS temperature guidelines are established by using applicable heat resistance information, heat penetration data, process calculations, and confirmation steps. Despite regulation, no test is currently available to verify temperature process adequacy in meat products.

Part III

Materials and Methods

Strain Preparation and Culture Maintenance

Four strains of nalidixic acid resistant *Salmonella* were used for this study: S. Hadar, S. Typhimurium, S. Montevideo, and S. Senftenburg (ATCC 43845). Each strain was stored at 4 °C on Tryptic Soy Agar slants (TSA; Difco; Becton Dickson; Sparks, MD). Cultures were transferred onto new slants monthly to maintain culture viability. The cultures used for this study were prepared as nalidixic acid resistant.

Nalidixic acid (Sigma-Aldrich Co; St. Louis, MO) solutions (200, 150, and 100 N) were prepared by diluting the anhydrous acid with sterilized, deionized water to the desired concentration level. The nalidixic acid solutions were filter sterilized and stored at 4 °C. Tryptic Soy Broth with nalidixic acid (TSBN) was prepared by adding 10 ml/ liter of each nalidixic acid concentration to sterile Tryptic Soy Broth (TSB; Difco; Becton Dickson; Sparks, MD). Each TSBN solution was mixed approximately 2 minutes and stored at 4 °C.

Each bacterial strain was aseptically inoculated into 6-7 ml of 100N TSBN and grown for 18-24 hrs at 35 °C. The process was repeated using 150N and 200N TSBN solutions. 200N TSBN cultures were used in each repetition of the experiment. Each bacteria strain was stored at 4 °C on TSA slants. Cultures were transferred onto new TSA slants monthly to maintain culture viability.

Microbial Analysis of Ground Beef

The ground beef from each cooking trial was prescreened for aerobic microorganisms and *Salmonella* according to the FDA BAM Manual (1997) with the

following modifications. 10 g of ground beef from each fat level was combined with 90 ml sterile .01% peptone water (Difco; Becton Dickson; Sparks, MD). The mixtures were agitated by hand in sterile Whirl-pak ® filter bags (Nasco; Fort Atkinson, WI) for approximately 2 minutes. Serial dilutions of the mixture were surface-plated onto Xylose Lysine Tergitol 4 agar plates (XLT4; Difco; Becton Dickson; Sparks, MD) and Plate Count Agar plates (PCA; Difco; Becton Dickson; Sparks, MD). The plates were incubated at 35 °C for 18-24 hours. 3 to 5 typical XLT4 colonies were inoculated into Triple Sugar Iron agar slants (TSI; Difco; Becton Dickson; Sparks, MD) for 18-24 hours at 35 °C. Samples that tested TSI –positive were tested with polyvalent-O *Salmonella* antiserum (Difco; Becton Dickson; Sparks, MD). Samples exhibiting positive antiserum results were further tested using API 20E rapid test materials (Biomerieux; Durham, NC). Aerobic colony counts were performed on the PCA plates and represent the total aerobic bacterial count.

Inoculation

Each strain of *Salmonella* was inoculated into 200 N TSBN and incubated at 35 °C for 18-24 hours. After growth, the 4 bacteria strains were mixed approximately 2 minutes to attain even distribution of each. Frozen ground beef was thawed 24 hrs prior to each cooking experiment. 3 fat levels (25%, 17%, and 10%) of ground beef were used. 9 g of ground beef from each fat level was inoculated with 1 g of the 4 strain bacteria cocktail to achieve a 1/10 dilution factor. Each sample was mixed for approximately 2 minutes to distribute the bacteria without changing the structure of the meat and each

sample was aseptically transferred into individual, sterile 13 x 100 mm slip-on cap thermal death tubes (Thermo Fisher Scientific Inc; Waltham, MA). The target inoculation level for the experiment was between 1og 7 and log 8 CFU/g.

Heating Procedure

The cooking tubes were placed into a metal rack. The water bath was heated by SAFGARD Pres-Vac Home Pasteurizer Model P-3000 (Schlueter Co; Jamesville, WI). The recording device was placed into the center of a non-inoculated sample. When the target temperature of the water was reached, samples were fully submerged into the cooking medium. Temperatures were recorded in 1 minute intervals for the total heating process. The target temperatures for the experiment were 60 °C, 65.6 °C, and 71.1 °C.

The temperature of the ground beef and heating water for Rep I 60 °C, 65.6 °C, and 71.1 °C experiments and Rep II 71.1° C experiment were monitored using a digital display data logger. Ground beef temperatures of Rep II 60 °C and 65.6 °C experiments and all ground beef temperatures of Rep III experiments were monitored with a Dickson HT100 High Temperature Logger (Dickson; Addison, IL). A Fisher Scientific Traceable ® thermocouple device was used to monitor and record the water bath temperatures of Rep II 60 °C and 65.6 °C experiments and all water bath temperatures of Rep III experiments.

At 60 °C, samples were removed at 0, 6, 12, 18, and 24 min. At 65.6 °C, samples were removed at 0, 4, 8, 12, and 16 min. At 71.1 °C for Rep I, samples were removed at 0, 3, 6, 9, and 12 min. At 71.1 °C for Rep II and Rep III, samples were removed at 0, 4, 6, 8, and 10 min. After removal from the water bath, samples were immediately cooled and

held in an ice bath. Non-inoculated tubes of ground beef were used as negative control samples and were removed at the beginning, middle, and end of the heating process.

Enumeration/ Identification

Enumeration was performed immediately after the heating procedure according to the FDA BAM Manual (1997) with the following modifications. Each sample was combined with 90 ml of 0.1% peptone water in a sterile Whirl-pak ® filter bag. The samples were agitated by hand for 2-3 minutes. The wash fluids were serial diluted and surface plated (0.1ml) onto XLT4 and PCA agars plates prepared with nalidixic acid at a .01 dilution factor. Plates were incubated at 35 °C for 18-24 hours. Colony counts were recorded for the PCA plates incubated at 35 °C for 18-24 hours. Typical black XLT4 colonies were inoculated onto TSI agar slants. Biochemical results were recorded. To determine the presence of *Salmonella*, all samples producing positive TSI slant results were further tested with polyvalent-O *Salmonella* antiserum. Antiserum-positive samples were tested using API 20E rapid test materials.

In order to validate complete destruction of *Salmonella*, sample enrichments were performed on samples that contained no growth at the experimental detection limit of log 2 CFU/g. 10 ml of each sample time that produced no growth were diluted with 90 ml of TSB and 5 ml of 200N nalidixic acid. Each enrichment solution was incubated at 35 °C for 18-24 hours. After incubation, each solution was streak plated onto XLT4 agar plates. Typical XLT4 colonies were inoculated into TSI agar slants and incubated for 18-24 hours at 35 °C. TSI –positive samples were tested with polyvalent-O *Salmonella* antiserum.

Polyvalent-O antiserum-positive samples were tested with *Salmonella* antigen-group-specific antiserums (Difco; Becton Dickson; Sparks, MD). 5 colonies from the PCA plates with nalidixic acid were tested with each antiserum and the number of group occurrences/ total colonies tested was recorded. S. Hadar, S. Typhimurium, S. Montevideo, and S. Senftenberg are members of antigen group C2, B1, C1, and E4 respectively.

Statistical Analysis

The survival of *Salmonella* (log colony forming unit [CFU]/ gram) was analyzed with SAS® 8.02 (SAS Institute; Cary NC). The statistical design of the experiment was completely randomized block design. The factorial treatments were temperature and fat. The significance of the factors was set at ($p < 0.05$) and Student-Newman-Kuels (SNK) method was used for mean separation.

Part IV

Results and Discussion

Introduction

The US Food Code and Code of Federal Regulations have established time and temperature requirements for comminuted meats and beef patties. The ideal process should meet the minimum heating requirements and reduce *Salmonella* bacteria by 6.5 logs CFU/g (Juneja and others 2001). In this experiment, fat levels of 10, 17, and 25% did not significantly affect microbial destruction ($p < 0.05$) at either 60, 65.5, of 71.1 °C heat treatments. Also, the most heat resistant serotype, S. Senftenburg, was identified after longer time periods of heat treatment more often than the other serotypes.

Heating Analysis

The heating curves for the ground beef samples during heat treatments in the water baths at 60, 65.6, and 71.1 °C are shown in figures 1-3. The temperatures from each replication within each temperature treatment were not significantly different from each other ($p > 0.05$) therefore, only the average curves are shown. As expected, higher water bath temperatures resulted in more rapid heating and higher temperatures of the ground beef samples.

The maximum temperature of the ground beef sample during the 60 °C heat treatment was 60.3 °C. The temperature of the ground beef samples at 6, 12, 18, and 24 minutes were 55.2, 59.8, 60, and 59.8 °C respectively. The temperature of the samples increased significantly from 0 minutes to 7 minutes and remained constant for the remaining 9 minutes of heat treatment. The heat treatment for rep III was significantly different from rep I and rep II . The temperature of the rep III samples increased at a

slower rate than the temperatures of the other repetitions and the rep III maximum temperature was lower than the maximum temperatures of the other repetitions

The 65.5 °C heat treatment resulted in a maximum temperature of the ground beef samples of 65.9 °C. The temperatures of the ground beef samples at 4, 8, 12, and 16 minutes were 55.6, 64.2, 65.6, and 65.9 °C respectively. The temperatures of the samples increased significantly from 0 minutes to 5 minutes and remained constant for the remaining 11 minutes. The heat treatment of rep III was significantly different from rep I and rep II. The temperature of the rep III samples increased at a slower rate than the temperatures of the other repetitions and the rep III maximum temperature was lower than the maximum temperatures of the other repetitions.

The maximum temperature of the ground beef sample during the 71.1 °C heat treatment was 70.0 °C. The temperatures of the ground beef samples at 3, 4, 6, 8, and 10 minutes were 55, 60.2, 64.9, 67.4, and 69.1 °C respectively. The temperature of the samples increased significantly from 0 minutes to 6 minutes and from 8 minutes to 9 minutes. After 9 minutes, the temperature did not increase significantly.

Microbial Analysis

The survival curves for the ground beef samples during heat treatments in the water baths at 60, 65.6, and 71.1 °C are shown in figures 13-15. The survival curves from each replication within each temperature treatment were not significantly different from each other ($p > 0.05$) therefore, only the average curves are shown. For the 60 °C heat treatment, *Salmonella* populations were 7.7, 7.1, 5.4, 4.3, and 4.1 logs CFU/g at 0, 6, 12, 18, and 24 minutes respectively. The *Salmonella* cocktail population decreased

significantly between 6 minutes and 18 minutes without significant decrease during the remaining 6 minutes of heating. The rep II survival curve was significantly different from rep's I, III. The Rep II heat treatment reached temperatures above 60 °C which resulted in significantly higher levels of microbial destruction.

The 65.6 °C heat treatment resulted in salmonella populations of 7.9, 7.0, 4.1, 2.1, and 2.0 logs CFU/g for 0, 4, 8, 12, and 16 minutes respectively. The *Salmonella* cocktail population decreased significantly between 0 minutes and 12 minutes and did not significantly decrease during the remaining 4 minutes of heating. The Rep I survival curve was significantly different from the survival curves of Rep's II, III. The Rep I heat treatment reached temperatures above 65.6 °C which resulted in significantly higher levels of microbial destruction.

The 71.1 °C heat treatments resulted in *Salmonella* cocktail populations of 7.4, 6.7, 4.5, 2.0, 2.0, and 2.0 logs CFU/g for 0, 4, 6, 8, 10, and 12 minutes respectively. The salmonella cocktail population decreased significantly between 0 minutes and 8 minutes and did not significantly decrease during the remaining 4 minutes of heating.

Predicted lethality and predicted log reductions for the ground beef samples during heat treatments in the water baths at 60, 65.6, and 71.1 °C are shown in tables 5-7. The predicted log reductions were 1.5, 6.4, and 8.8 logs for the 60, 65.6, and 71.1 °C heat treatments. Actual log reductions for 60, 65.6 °C were similar to the predicted values. The 71.1 °C actual log reduction was below the predicted value. The 71.1 °C microbial analysis was limited by the detectable limit of 2 logs CFU/g and no enrichment analysis of the last ground beef sample. The log reduction of 60 °C for 24 minutes was

comparable to the log reduction of 65.5 °C heat treatment for 8 minutes and the log reduction of 71.1 °C for 6 minutes. Also, 65.5 °C for 12 minutes resulted in similar log reduction results as 71.1 °C for 8 minutes.

Contrary to similar studies, fat content did not affect microbial destruction. Juneja and others 2000 found less microbial destruction of *Salmonella* cocktail in ground beef of higher fat content when heated between 58- 65 °C. According to the study, higher fat content resulted in higher sample come-up times and higher D values. Less destruction of *Salmonella* was also seen in ground beef of higher fat levels in a study conducted by Smith and others 2001. Ahmed and other 1995, Faith and others 1998a saw less reduction of *E coli* in ground beef. Differences in sample size, product formulation, and heating conditions may have contributed to the differences between this study and other studies. 10g sample sizes were used, which may have affected heating conditions. Also, the ground beef was formulated commercially and may have contributed to some differences between the samples of this study and others.

Serotype Analysis

Serotype distributions in the ground beef samples during heat treatments in the water baths at 60, 65.6, and 71.1 °C are shown in figures 25-42. During the initial stages of heat treatment, the 4 serotypes were evenly distributed throughout all ground beef samples. The probability of detection was equal among all serotypes (25%). As expected, S. Senftenberg was found more often at the later stages of heat treatment of 65.5 °C (8, 12, and 16 minutes) and 71.1C (6, 8, 9, and 10 minutes). S. Senftenberg was the only serotype identified through enrichment of the heat samples where no growth was

observed at the detectable limit of 2 logs CFU/g. These results are in agreement with other studies that have used S. Senftenburg. Doyle and others 2000 reported S. Senftenburg as the most heat resistant *Salmonella* serotype. Heat inactivation studies of *Salmonella* cocktails in ground beef by Smith and others 2001, Orta-Ramirez and others 1997 also reported S. Senftenburg as the serotype with higher D values, relating to more heat resistance when compared to the other serotypes used.

Part V

Summary

Summary

Based on the reduction of *Salmonella* cocktail, the most effective heat treatment is 60 °C for 12 to 16 minutes. This heat treatment reduced the bacterial population 5.9 logs/ CFU, which is greater than the heat treatment of 71.1 °C for 8 to 10 minutes. Industry can utilize cooking combinations at lower temperatures to maintain sensory characteristics and significantly reduce pathogens. If the average bacterial populations were higher, the heat treatments would possibly have bacterial reductions that are greater than USDA regulation of 6.5 logs. Due to fat not being a significant factor in bacterial reduction, industry could establish heat processes that `can be used on all beef patties regardless of fat composition. This could reduce processing time and reduce operating costs overall.

According to the serotype data and enrichment data, S. Senftenburg was found most often during longer exposure to the heat treatments. If the colonies tested for serotype groups are used to represent the entire population percentage, S. Senftenberg is the most heat resistant This suggests that only S. Senftenburg should be used in heat resistance studies instead of the use of multiple- strain cocktails. Although S. Senftenberg is seen more in pork products, accounting for only the most resistant strain could be a sufficient safety factor in microbial studies and could increase accuracy.

According the enrichment data, S. Senftenberg contained in the ground beef samples could not be destroyed by heat to levels below 2 log CFU/g. As a result, the heat resistance of S. Senftenberg reduces the total microbial destruction of the heating experiments. In the absence of S. Senftenberg, the complete microbial destruction of the *Salmonella* cocktail could have been achieved. Therefore the presence of this heat

resistant strain affected both the rate of microbial destruction and the total microbial destruction.

Although there are visible differences in the level of microbial destruction as the fat levels of the samples were adjusted, no significant differences in microbial destruction could be attributed to fat level. Significant differences due to fat level may have been observed in this experiment without the use of S. Senftenberg. S. Senftenberg reduced the level of total microbial destruction in the samples because it could not be completely destroyed. High variability of the heat penetration data was also observed. Less variability of the heating data could result in significant differences in microbial destruction that can be attributed to changes in fat levels.

Differences in the sample size and preparation of the ground beef samples could result in the differences in the microbial destruction in ground beef of this experiment when compared to other experiments. Sample sizes of other studies range from 1-150 grams. This experiment used 10 g samples. This sample size is within the range of other studies and the thickness of the samples was similar to restaurant hamburgers. However, the size of the samples could significantly affect the overall microbial destruction results of the experiment.

Preparation of the ground beef before use in the heat study is also a factor that could affect the final results of the experiment. Other studies may have prepared the different fat levels of the ground beef by various methods and commercial equipment. The shear and speeds of processing equipment will determine the amount of mixing and the level of emulsion between the fat, proteins, and water in the ground beef. Ground beef

that has been mixed at higher levels of shear and speed could possess higher levels of emulsion characteristics. Higher levels of emulsions will result in more water entrapment of the ground beef as it is heated. The bacteria could survive higher temperatures and longer heating periods if moisture remains in the ground beef which has a higher level of emulsion.

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Appendix

Table 1: Six most common *Salmonella* serotypes isolated from large ground beef producing HACCP plants January 2, 1998 to January 25, 1999 (FSIS 2002c).

Rank	Serotype	Percentage
1	Anatum	19 %
2	Hadar	11.9 %
3	Muenster	11.9 %
4	Meleagridis	9.5 %
5	Typhimurium (var. Copenhagen)	9.5 %
6	Montevideo	7.1 %
7-19	Various	31.1 %

Table 2**:** *Salmonella* Performance Standards (US Government Print Office 2007a, e)

Product	Allowable %	Number of Samples	Maximum Positive Samples Allowed
Steers/ Heifers	1.0	82	1
Cows/Bulls	2.7	58	2
Ground Beef	7.5	53	5
Hogs	8.7	55	6
Broilers	20	51	12
Ground Chicken	44.6	53	26
Ground Turkey	49.9	53	29

Table 3: US Food Code 3-401.11 Minimum Time, Temperature Requirements for Raw Animal Foods (FDA 2006).

Temperature °C	Time
63	3 minutes
66	1 minute
68	15 seconds
70	< 1 second (instantaneous)

Table 4: 9 CFR 318.23 Permitted Heat-Processing Temperature/Time Combinations for Fully Cooked Patties (US Government Print Office 2007c)

Internal Temperature °F	Time
151	41 seconds
152	32 seconds
153	26 seconds
154	20 seconds
155	16 seconds
156	13 seconds
157	10 seconds

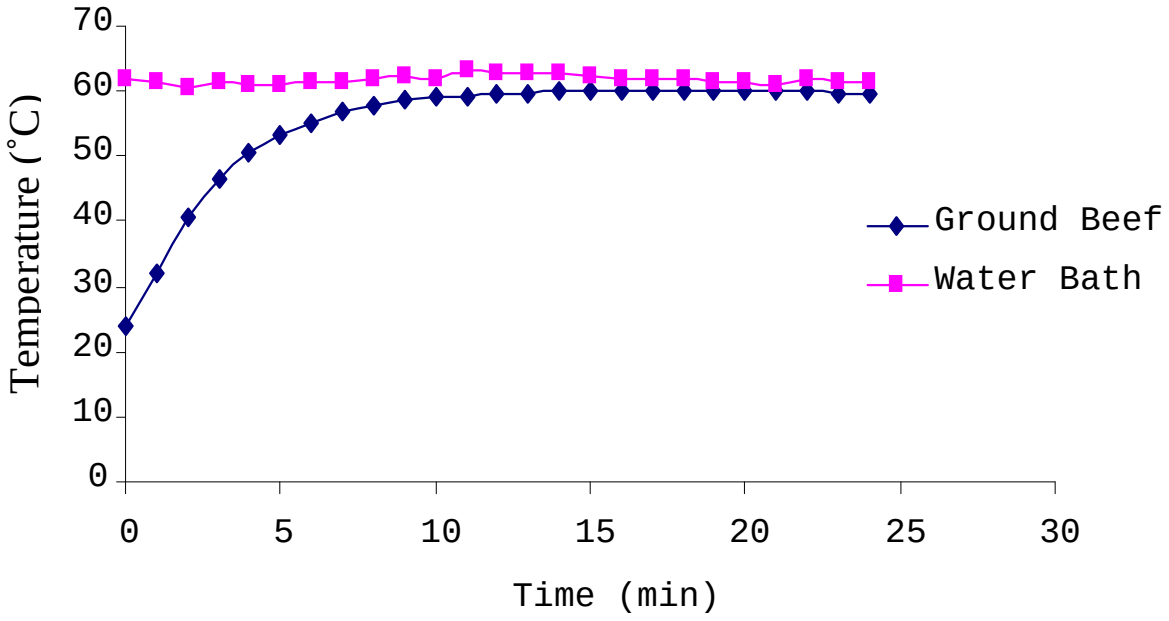

Figure 1: Average 24 Minute Heat Treatment of 10, 17, and 25% Fat Ground Beef Samples in Water Bath at Target Temperature of 60°C

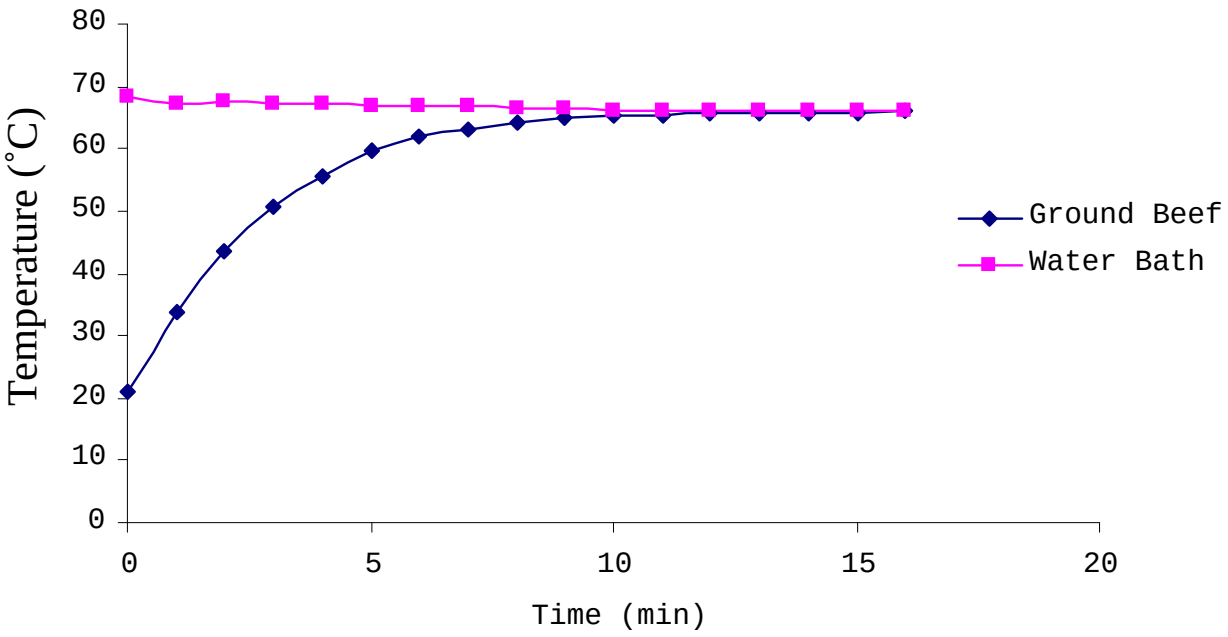

Figure 2: Average 16 Minute Heat Treatment of 10, 17, and 25% Fat Ground Beef Samples in Water Bath at Target Temperature of 65.6°C

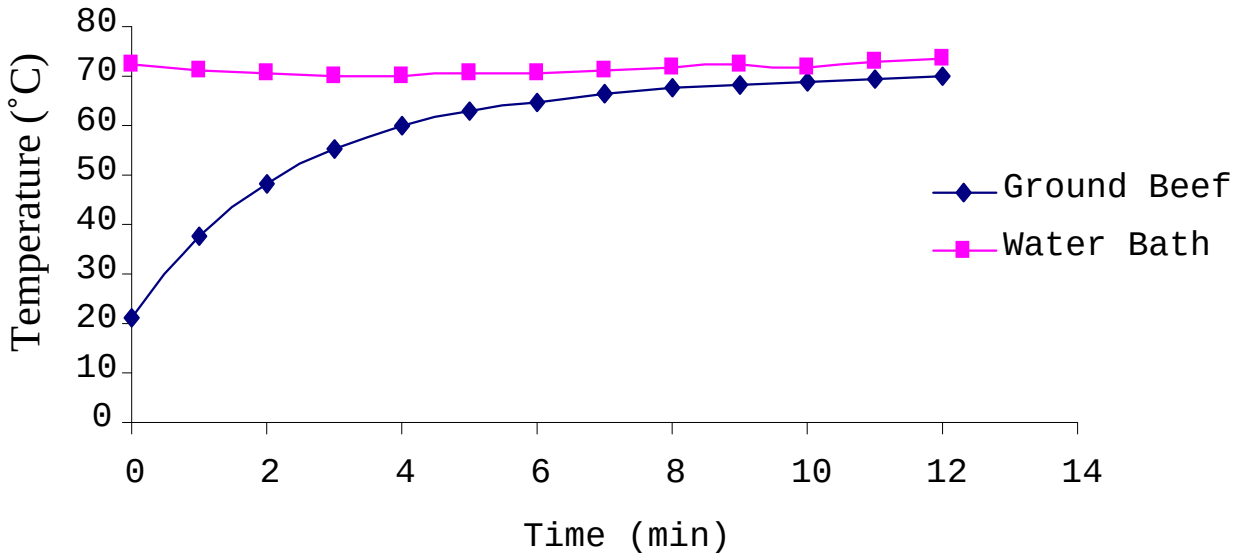

Figure 3: Average 12 Minute Heat Treatment of 10, 17, and 25% Fat Ground Beef Samples in Water Bath at Target Temperature of 71.1°C

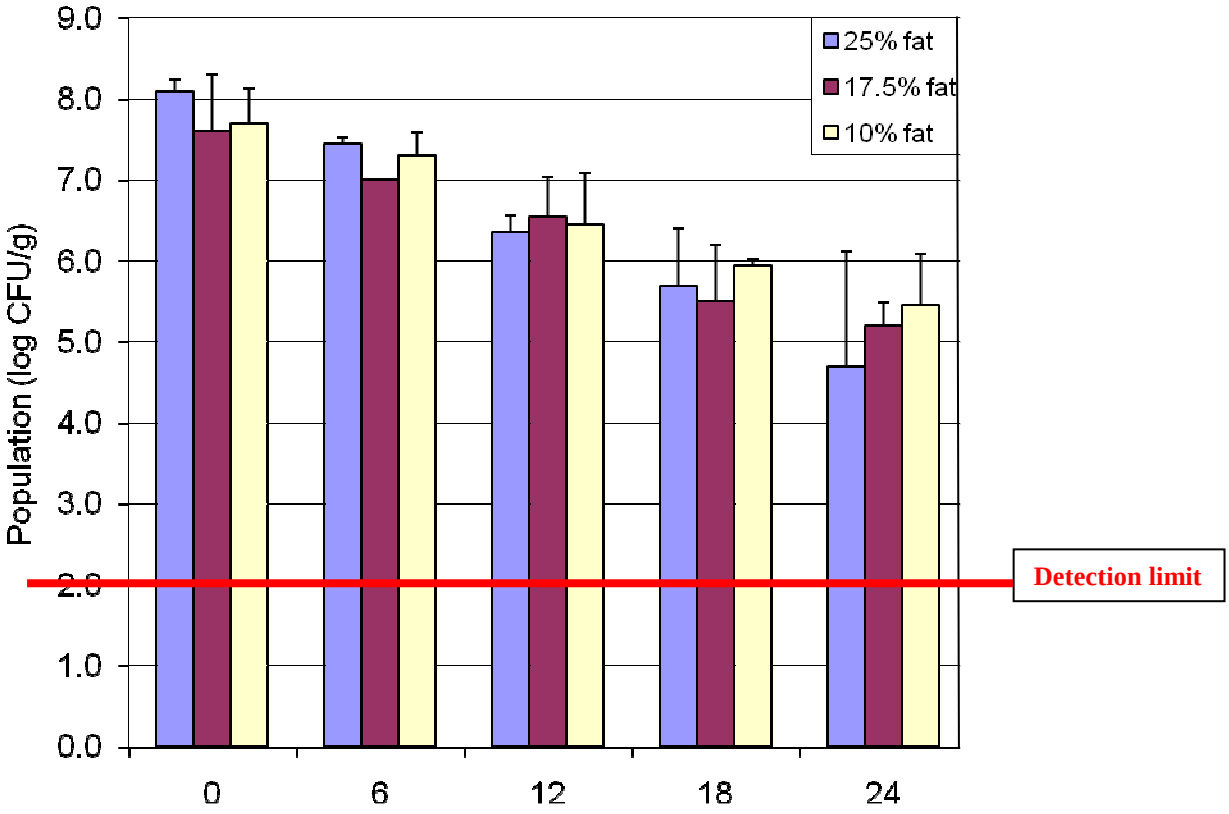

Figure 4. Average Survival of Salmonella Cocktail Inoculated into 10, 17, and 25% Fat Ground Beef Heated for 24 Minutes in a Water Bath to a Target Temperature of 60 °C

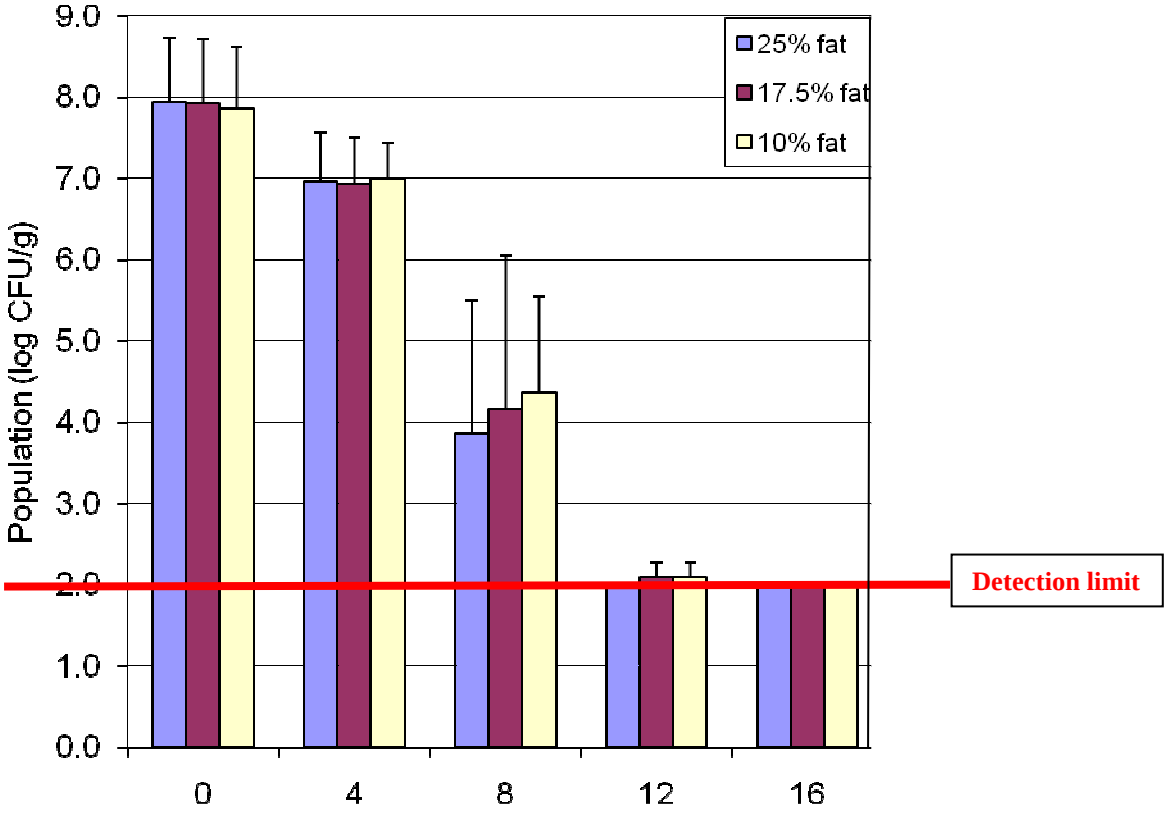

Figure 5. Average Survival of Salmonella Cocktail Inoculated into 10, 17, and 25% Fat Ground Beef Heated for 16 Minutes in a Water Bath to a Target Temperature of 65.6 °C

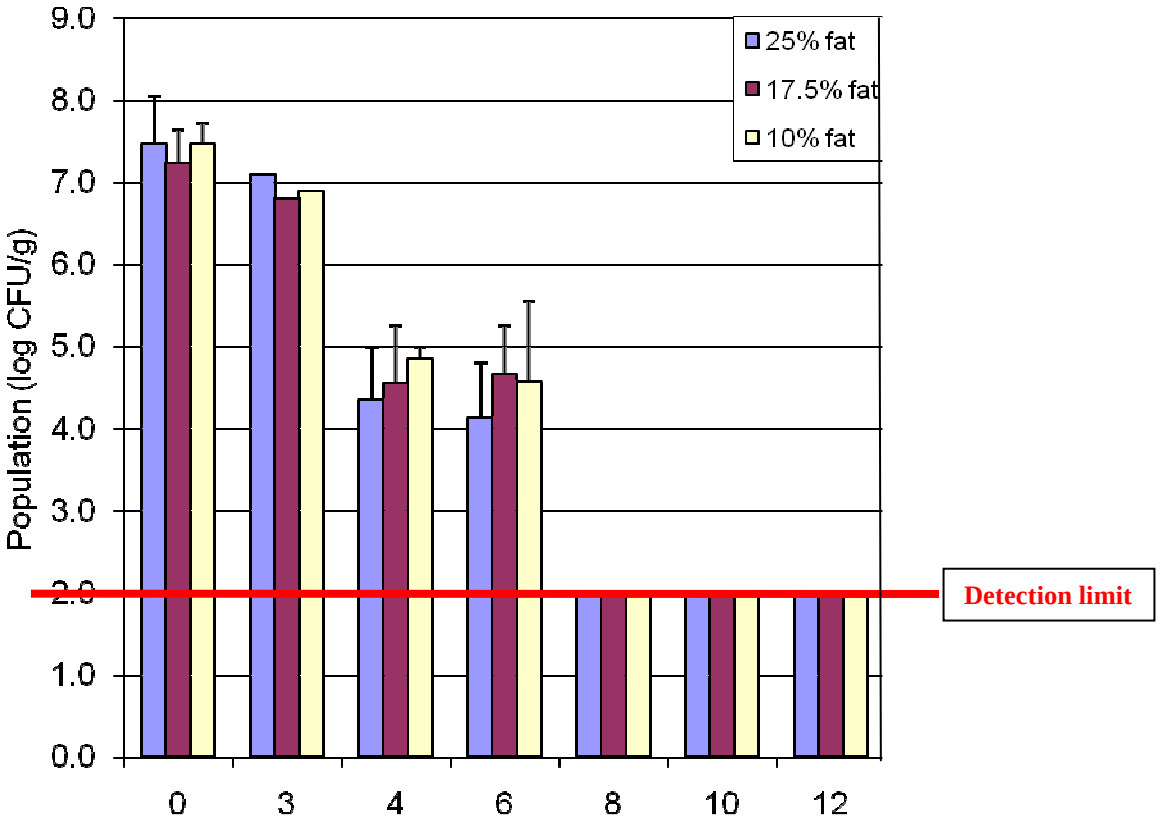

Figure 6. Average Survival of Salmonella Cocktail Inoculated into 10, 17, and 25% Fat Ground Beef Heated for 12 Minutes in a Water Bath to a Target Temperature of 71.1 °C

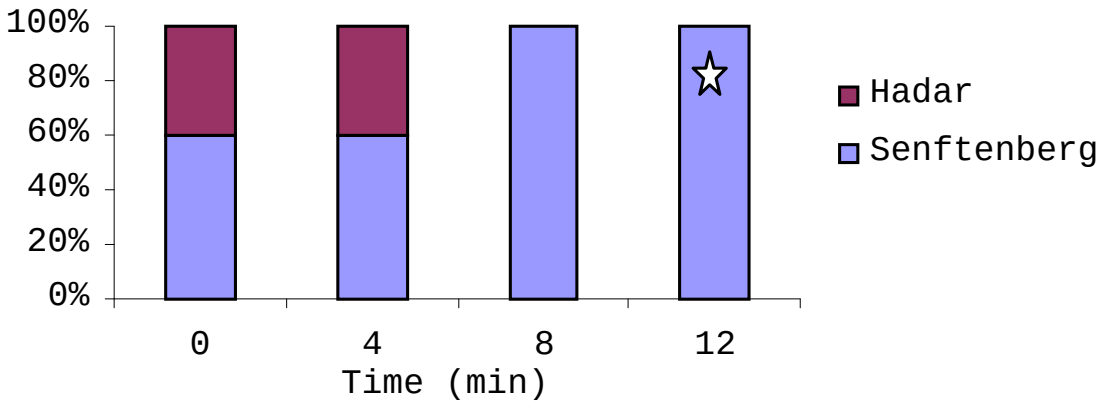

Figure 7. Rep 1 Distribution of Recovered Salmonella Serotypes in 25% Fat Ground Beef Samples Heated to 65.6°C over 16 minutes; * = enrichment

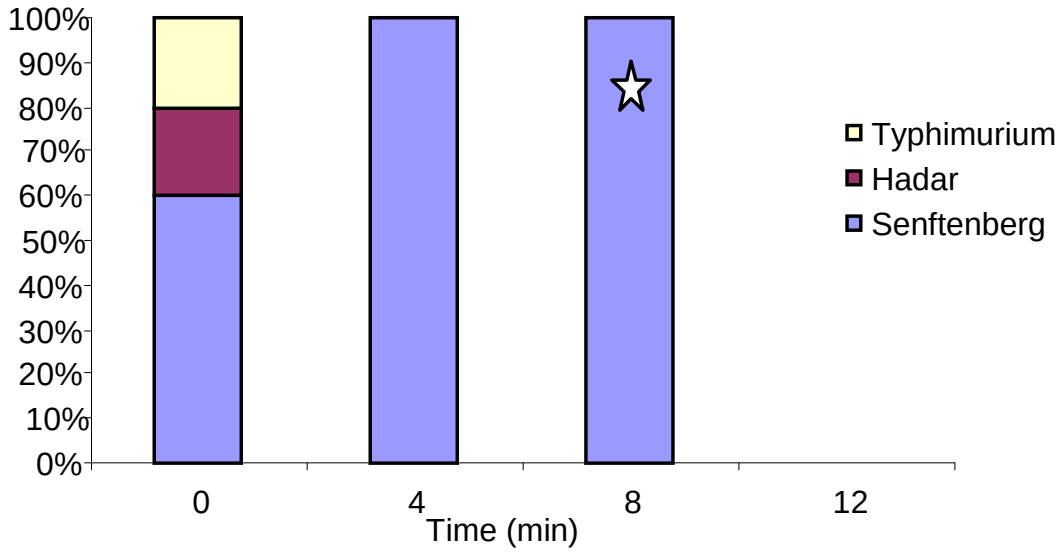

Figure 8: Rep 1 Distribution of Recovered Salmonella Serotypes in 17% Fat Ground Beef Samples Heated to 65.6°C over 16 minutes; * = enrichment

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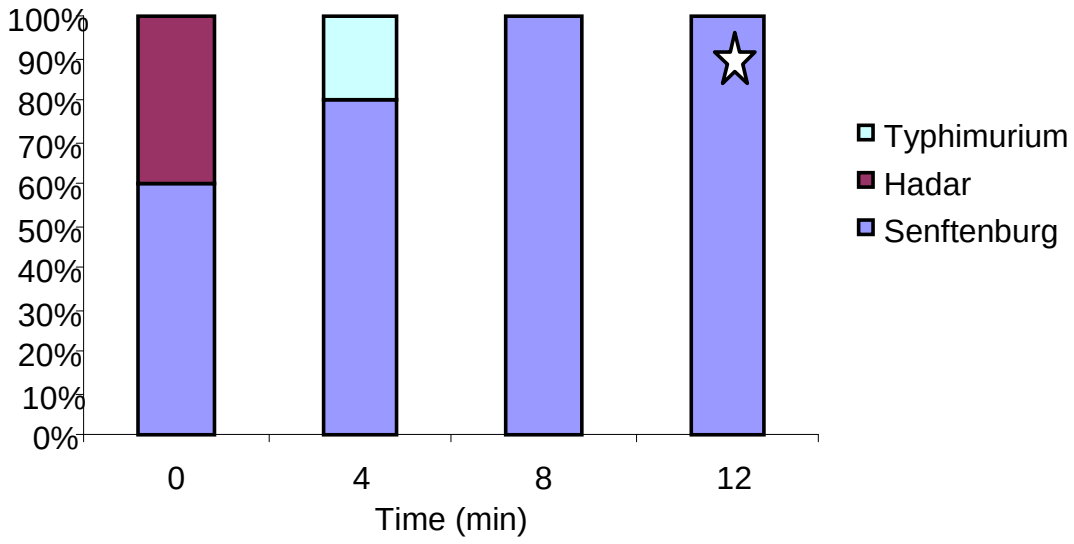

Figure 9. Rep 1 Distribution of Recovered Salmonella Serotypes in 10% Fat Ground Beef Samples Heated to 65.6°C over 16 minutes; * = enrichment

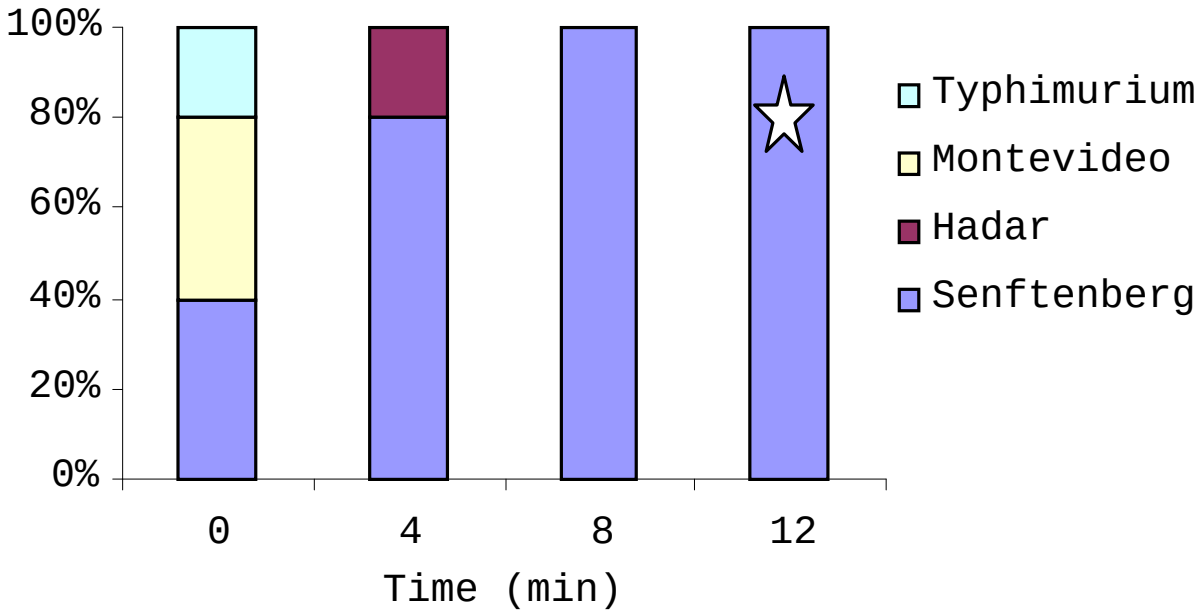

Figure 10. Rep 2 Distribution of Recovered Salmonella Serotypes in 25% Fat Ground Beef Samples Heated to 65.6°C over 16 minutes; * = enrichment

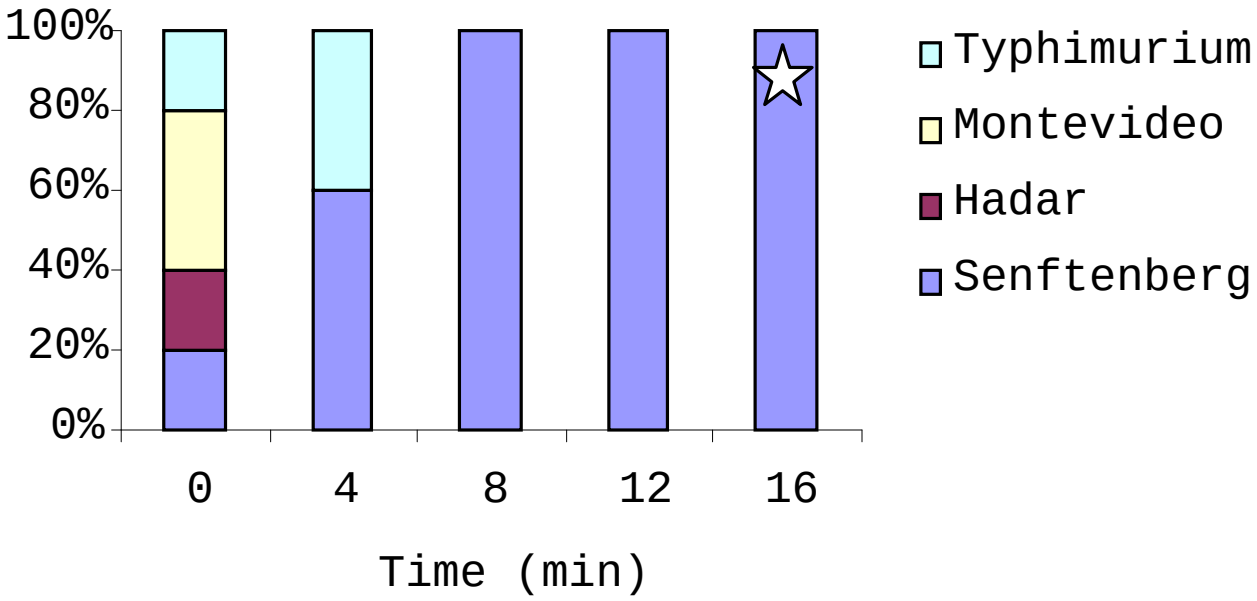

Figure 11: Rep 2 Distribution of Recovered Salmonella Serotypes in 17% Fat Ground Beef Samples Heated to 65.6°C over 16 minutes; * = enrichment

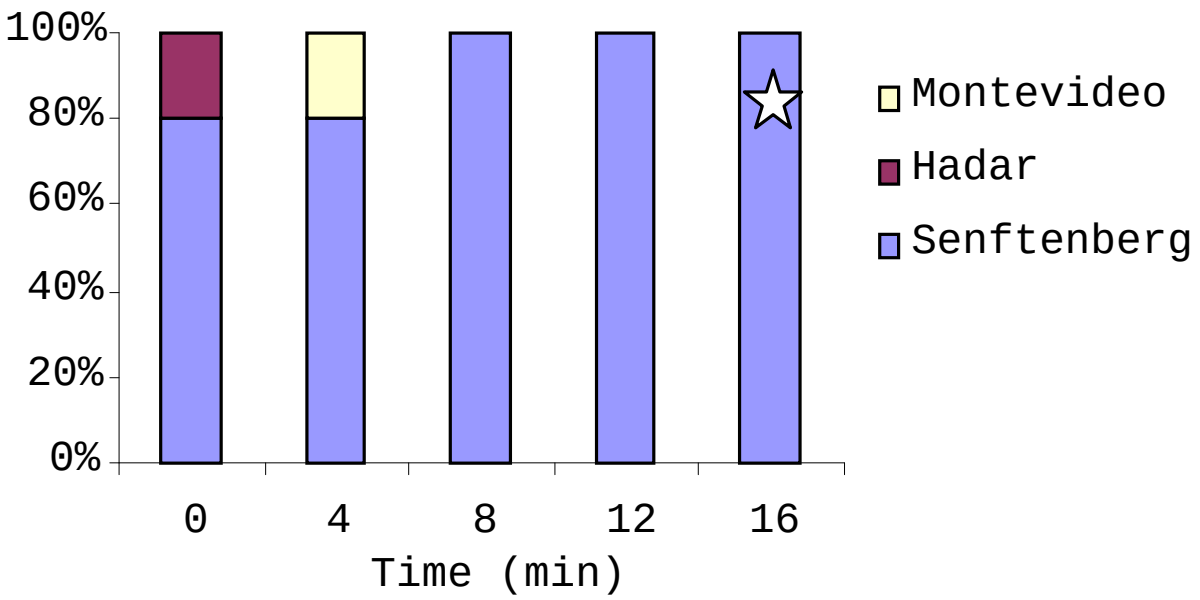

Figure 12. Rep 2 Distribution of Recovered Salmonella Serotypes in 10% Fat Ground Beef Samples Heated to 65.6°C over 16 minutes; * = enrichment

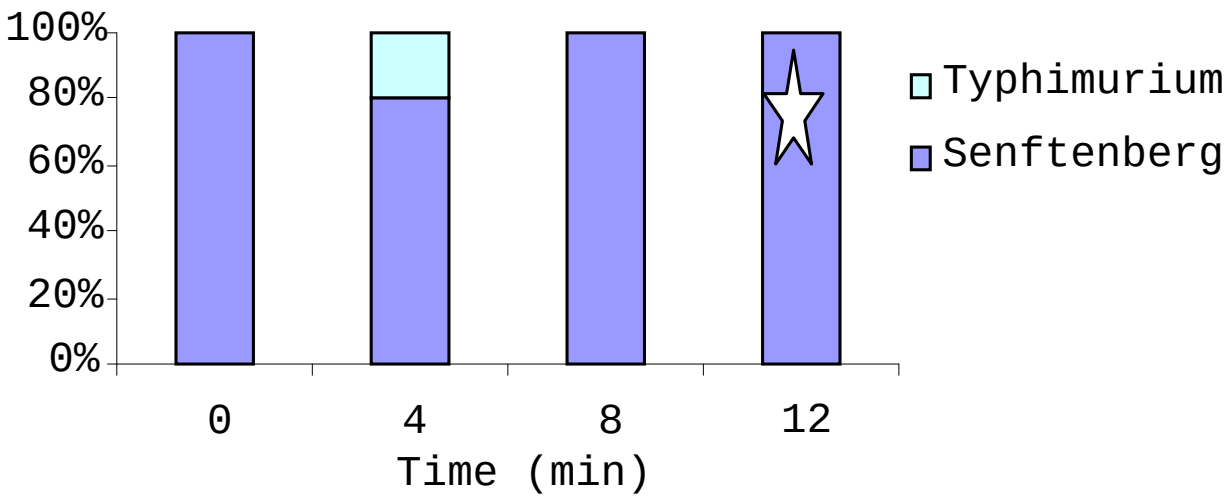

Figure 13. Rep 3 Distribution of Recovered Salmonella Serotypes in 25% Fat Ground Beef Samples Heated to 65.6°C over 16 minutes; * = enrichment

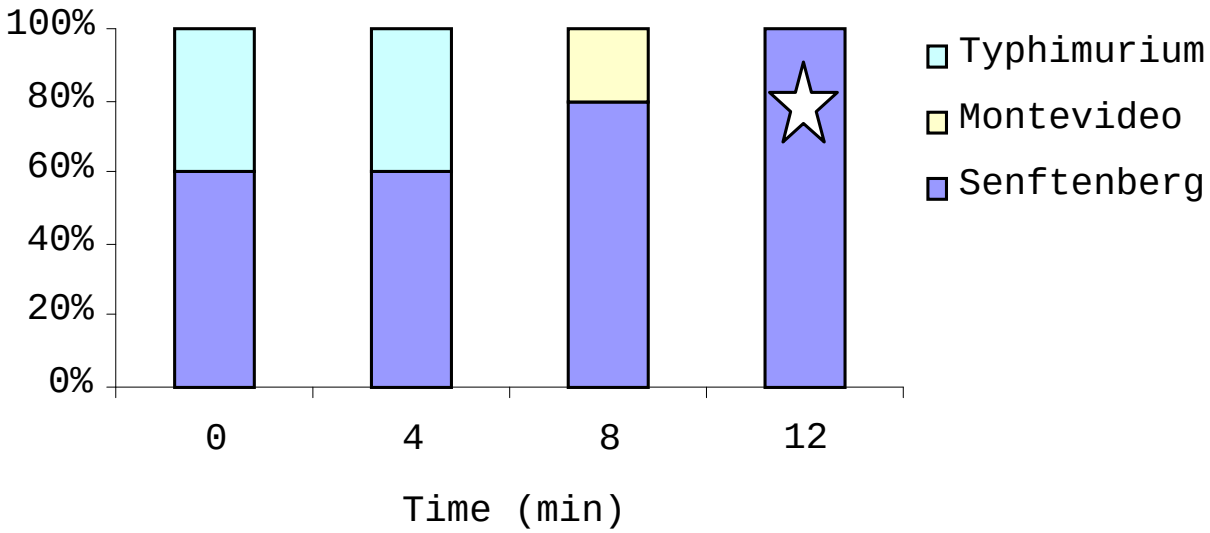

Figure 14. Rep 3 Distribution of Recovered Salmonella Serotypes in 17% Fat Ground Beef Samples Heated to 65.6°C over 16 minutes; * = enrichment

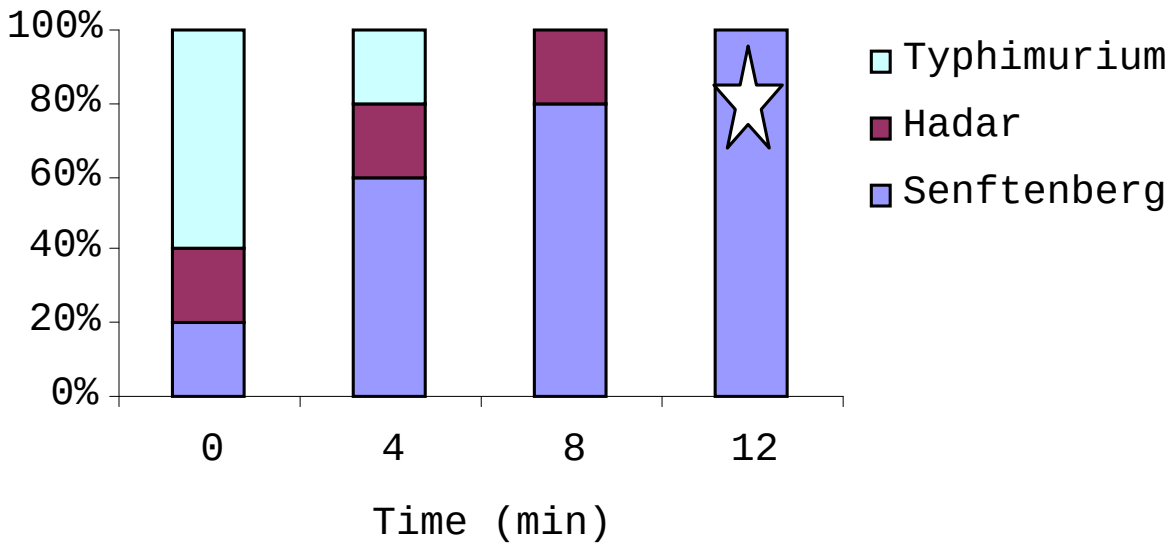

Figure 15.Rep 3 Distribution of Recovered Salmonella Serotypes in 10% Fat Ground Beef Samples Heated to 65.6°C over 16 minutes; * = enrichment

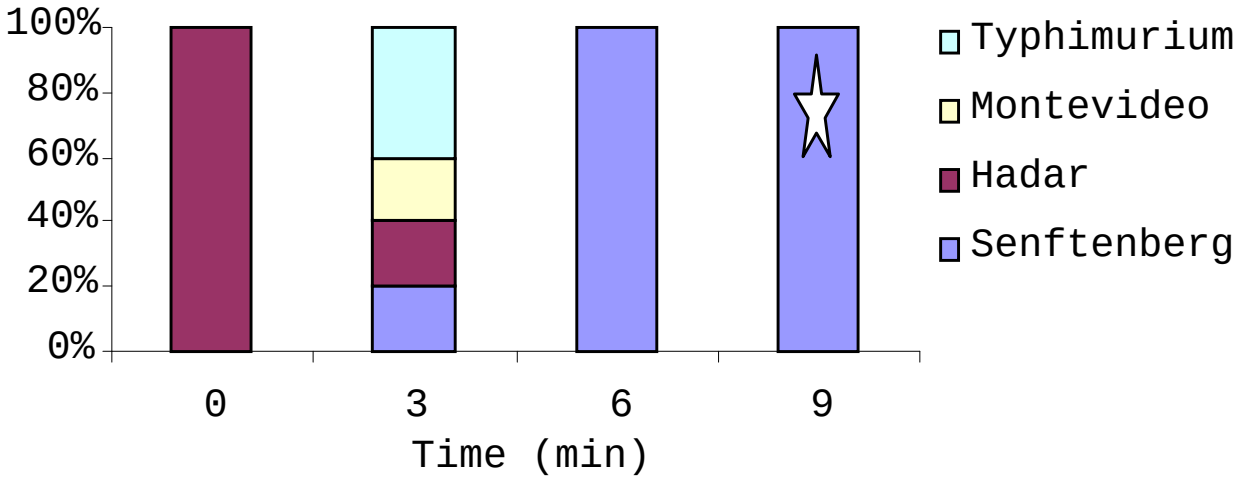

Figure 16. Rep 1 Distribution of Recovered Salmonella Serotypes in 25% Fat Ground Beef Samples Heated to 71.1°C over 12 minutes; * = enrichment

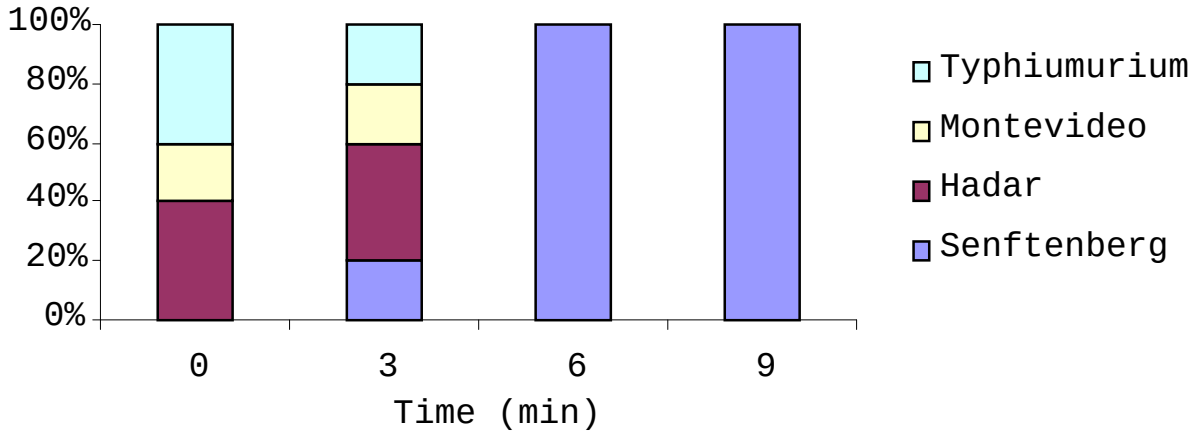

Figure 17. Rep 1 Distribution of Recovered Salmonella Serotypes in 17% Fat Ground Beef Samples Heated to 71.1°C over 12 minutes

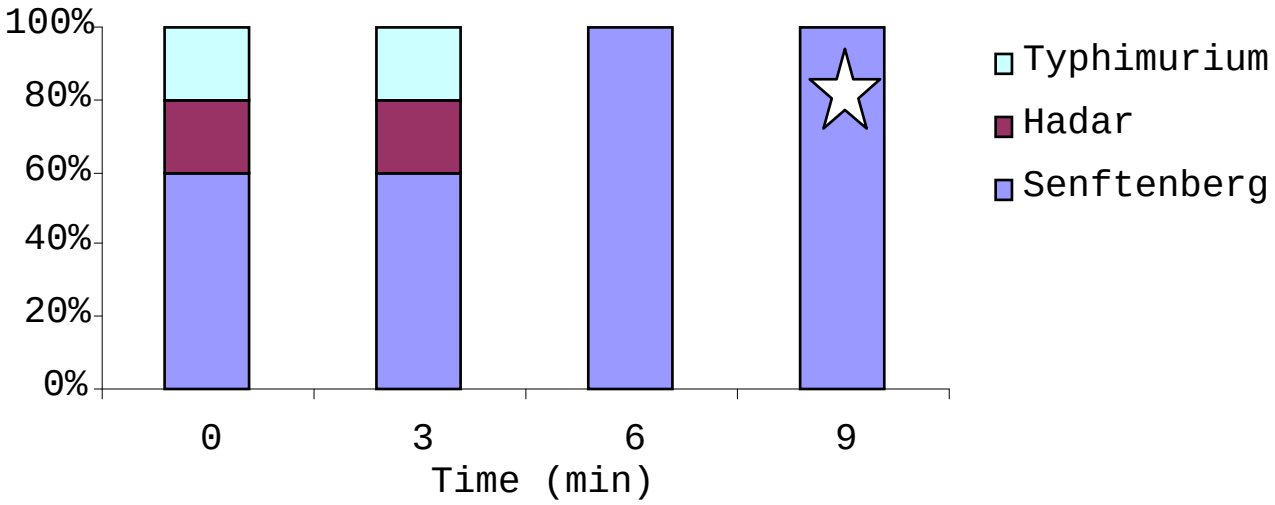

Figure 18. Rep 1 Distribution of Recovered Salmonella Serotypes in 10% Fat Ground Beef Samples Heated to 71.1°C over 12 minutes; * = enrichment

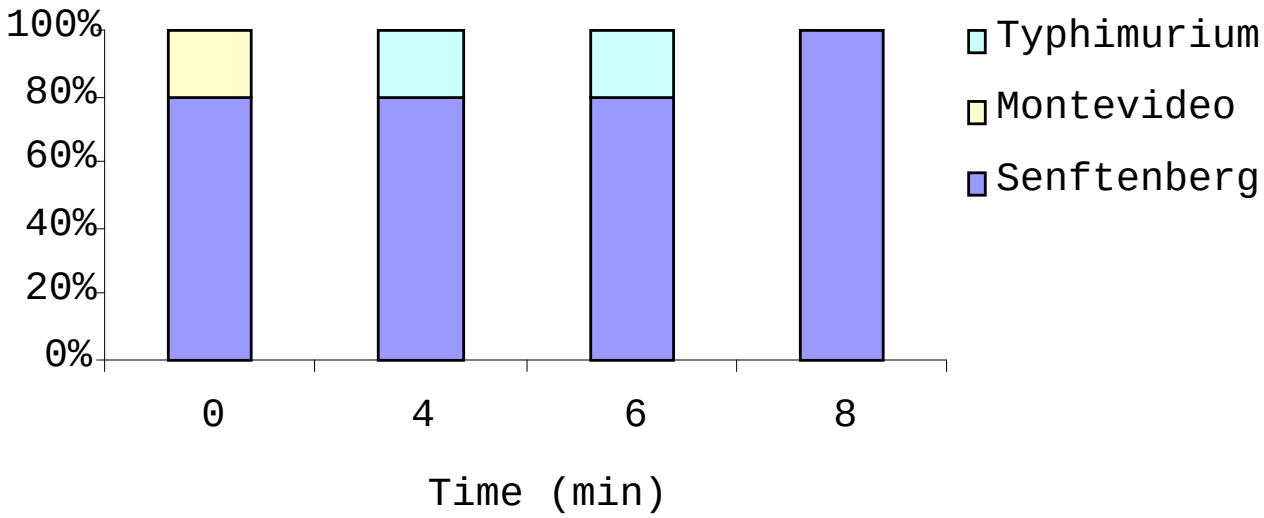

Figure 19. Rep 2 Distribution of Recovered Salmonella Serotypes in 25% Fat Ground Beef Samples Heated to 71.1°C over 10 minutes

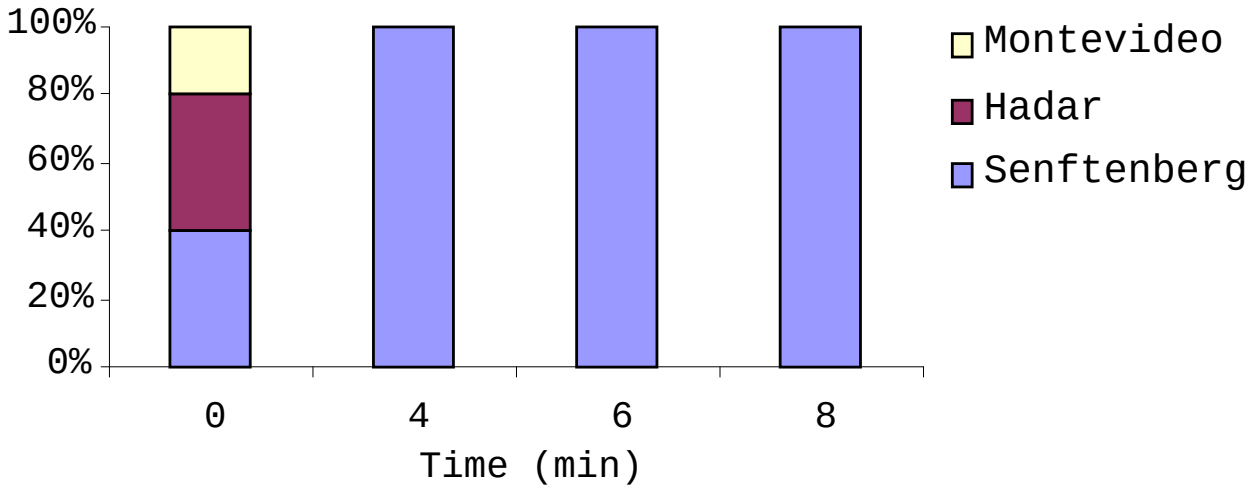

Figure 20. Rep 2 Distribution of Recovered Salmonella Serotypes in 17% Fat Ground Beef Samples Heated to 71.1°C over 10 minutes

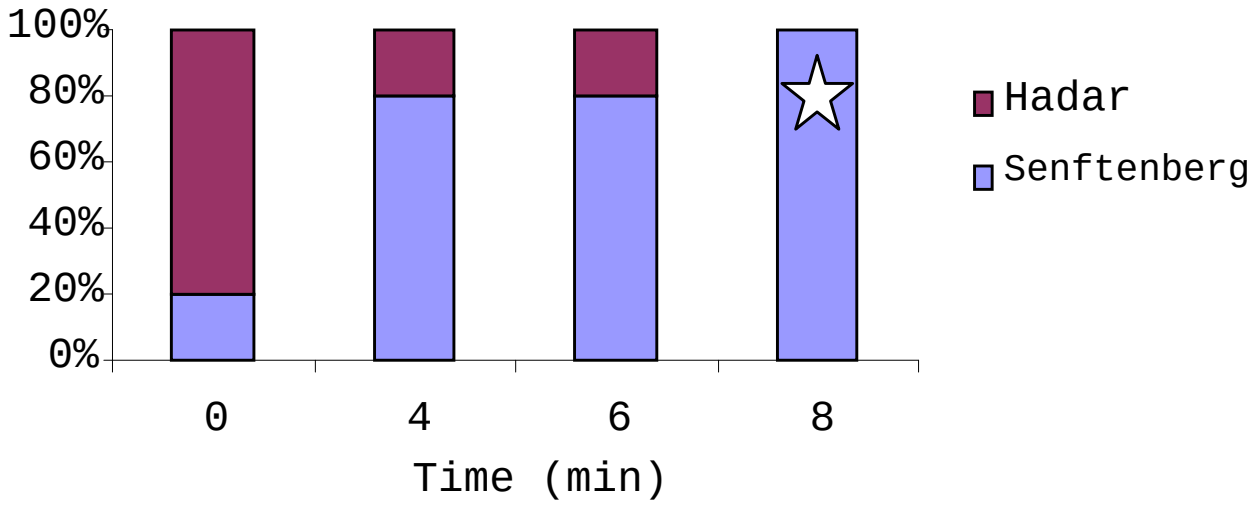

Figure 21. Rep 2 Distribution of Recovered Salmonella Serotypes in 10% Fat Ground Beef Samples Heated to 71.1°C over 10 minutes; * = enrichment

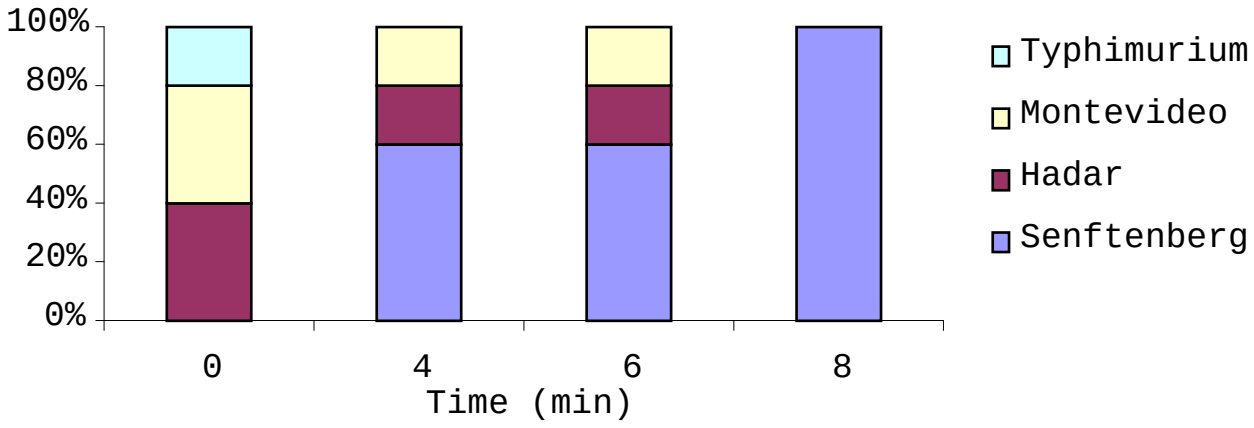

Figure 22. Rep 3 Distribution of Recovered Salmonella Serotypes in 25% Fat Ground Beef Samples Heated to 71.1°C over 10 minutes

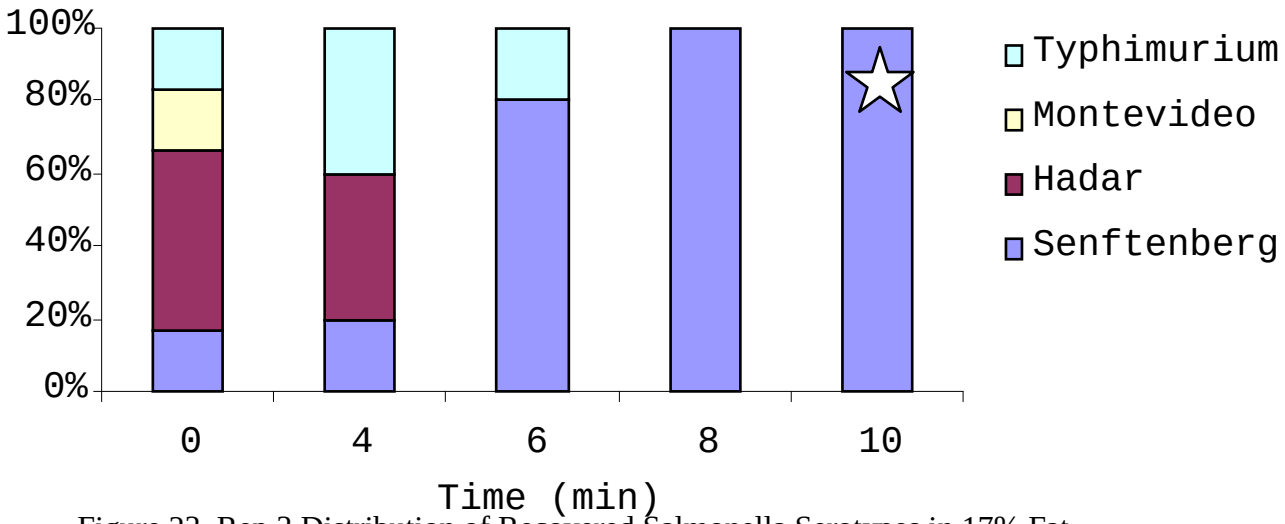

Figure 23. Rep 3 Distribution of Recovered Salmonella Serotypes in 17% Fat Ground Beef Samples Heated to 71.1°C over 10 minutes; * = enrichment

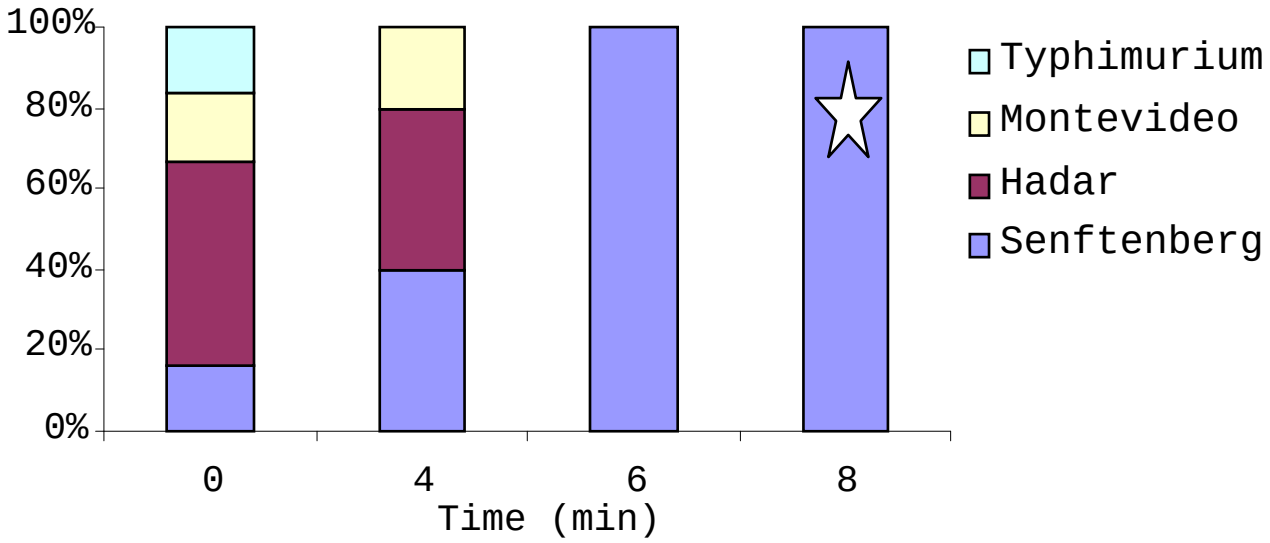

Figure 24. Rep 3 Distribution of Recovered Salmonella Serotypes in 10% Fat Ground Beef Samples Heated to 71.1°C over 10 minutes; * = enrichment

Table 5 : Predicted F value and Log Reduction for 71.1C Heat Treatment over 10 minutes

Time (min)	Cumulative F value Reduction (min/ log)	Cumulative Log Reduction (log/ CFU)	Actual Log Reduction (log/ CFU)
0	0.000	0.00	0.00
4	0.012	0.05	0.73
6	0.179	0.82	2.93
8	0.703	3.20	5.39
10	1.943	8.83	5.39

Table 6: Predicted F value and Log Reduction for 65.6 C Heat Treatment over 16 minutes

Time (min)	Cumulative F value (min/ log)	Cumulative Log Reduction (log/ CFU)	Actual Log Reduction (log/ CFU)
0	0.000	0.00	0.00
4	0.004	0.02	0.94
8	0.197	0.90	3.78
12	0.745	3.39	5.84
16	1.399	6.36	5.91

Table 7: Predicted F value and Log Reduction for 60 C Heat Treatment over 24 minutes

Time (min)	Cumulative F value (min/ log)	Cumulative Log Reduction (log/ CFU)	Actual Log Reduction (log/ CFU)
0	0.000	0.00	0.00
6	0.008	0.04	0.60
12	0.103	0.47	2.37
18	0.233	1.06	3.44
24	0.338	1.53	3.65

Vita

Christopher Jerome Williams was born in Mobile, Ala on August 6, 1975. He was raised in Memphis, TN and went to St. Ann Grade School in Bartlett, TN. He graduated from Gonzaga High School in 1993. From there, he went to the University of Tennessee Knoxville. He received a B.S. in food science and technology in 1999, a B.S. in accounting in 2007, and a M.S. in food science and technology in 2009.

Chris is currently working in the food industry and attaining business and accounting certifications.