Heat Resistance of *Escherichia coli* and *Salmonella enterica* in Ground Beef and Chicken

by

Jordan Brad Webster

A thesis submitted in partial fulfillment of the requirements for the degree of

Master of Science

in

Food Science and Technology

Department of Agricultural, Food and Nutritional Science
University of Alberta

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Abstract

Escherichia coli and *Salmonella* are contaminants in meat products and pose a risk for foodborne illness. Thermal lethality is the traditional method for controlling pathogens in meat products. It is recommended that ground beef and poultry products be cooked to internal temperatures of 71°C and 74°C, respectively. However, *E. coli* and *Salmonella* have a wide variance in resistance to heat. The aim of the current research was to determine the heat resistance of heat-resistant and heat-sensitive *E. coli* and *Salmonella* in ground beef and chicken and to determine the influence of the presence of a seasoning binder on heat resistance in meat products. Ground beef with and without 5.6% seasoning binder was inoculated and heated at temperatures between 55 and 70°C for different times. A linear regression model was used to calculate the *D*- and *z*-values. Seasoning binder increased the decimal reduction times (*D*-values) of heat-resistant *E. coli* AW1.7 but not of heat-resistant *S. enterica* ATCC 43845 in ground beef. To determine inactivation in patties, ground beef with and without 5.6% seasoning binder was inoculated with heat-resistant and heat-sensitive *E. coli* and *Salmonella.* When cooked to an internal temperature of 71°C, the presence of seasoning binder did not increase survival of heat-resistant or heat-sensitive *E. coli* and *Salmonella* in ground beef patties. Cooking ground beef patties to an internal temperature of 74°C achieved greater than 5-log (CFU/g) reduction of heat resistant *E. coli* AW1.7. The addition of 5.6% seasoning binder increased the survival of a cocktail of verotoxigenic *E. coli* inoculated in ground beef patties cooked to an internal temperature of 71°C. The cocktail was only reduced 2.8 log (CFU/g) in ground beef patties cooked to 71°C. To determine thermal inactivation in chicken nuggets, ground chicken was inoculated with heat-resistant and heat-sensitive *E. coli* and *Salmonella,* formed into chicken nuggets, heated and frozen. After being cooked to an internal temperature of 74°C cell counts of heat-resistant *E. coli*

and *Salmonella* were reduced less than 3-log (CFU/g) and heat-sensitive *E. coli* were reduced by greater than 5-log (CFU/g). In conclusion, recommended cooking guidelines are not sufficient to prevent the survival of heat-resistant *E. coli* and *Salmonella*. The results of this study can be used by producers to help to assess their process and predict the survival of heat resistant organisms in ground beef and chicken products.

Dedication

To my life-advisor, my late grandfather Charles Daryl Webster: because I owe it all to you. Many Thanks!

Acknowledgements

Four years ago I was living in my car while working at a slaughterhouse and suffering from a parasite from a contaminated water source. Through some twist of fate and love of food science I continued forward torturing myself by pursing grad school. I would like to give a tremendous amount of thanks to my advisor Dr. Lynn McMullen for all her help over the years. She was my introduction and guide through the wonderful and fascinating world of food microbiology. She is always there to believe in me as a student and give me confidence as a person. Additionally, I would like to thank Dr. Michael Gänzle for always offering his much-appreciated critical input and reminder of the need for a sexy story.

Thank you to my colleagues in 2-50 who were a constant source of help and encouragement. It was great sharing a laboratory with all of you during the last two years. Thank you to all those who came before me (some who were a part of the *E. coli* AW1.7 research tree) and who's work furthered our understanding of heat resistance. A notable thank you to Brian Walker and Chandré Van De Merwe who started grilling the burgers that kicked off my research.

I am grateful to my siblings and parents, who have provided me with moral and emotional support throughout my life. They showed me the comfort of a home and a hug.

A very special gratitude to the funding source NSERC Create – Meat Education and Training Network who's funding allowed me to develop skills and experience to be responsible in the meat industry. Cargill Inc. is acknowledged for supplying ground beef for this project.

Finally thank you, potentially unknown reader, for taking the time to read my thesis. I hope my work, best summarized as science of fast food cooking, provides you with interest, insight, or at least entertainment in the fact that it is about burgers and chicken nuggets.

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List of Symbols and Abbreviations

ANOVA	Analysis of variance
ATCC	American Type Culture Collection
a_w	Water activity
CFIA	Canadian Food Inspection Agency
CFU	Colony forming unit
LB	Luria-Bertani
LHR	Locus of Heat Resistance
Mfg aid	Manufacturing aid
ORF	Open Reading Frame
NRTE	Not Ready-to-Eat
RTE	Ready-to-Eat
TLPQC	Transmissible loci for protein quality control
VTEC	Verotoxigenic *Escherichia coli*

1. Introduction and Literature Review

Meat products, such as those from beef and chicken, are often the source of *Escherichia coli* and *Salmonella* infections. Ground beef is a critical source of *Salmonella* as it was implicated in 17 (45%) of the 38 outbreaks linked to beef reported during 2002–2011 in the United States (Laufer et al., 2015). The main source of pathogens on meat products is contamination during the slaughter and fabrication process as *E. coli* and *Salmonella* are present in the gut of livestock animals. Despite numerous efforts by processors during slaughter, including interventions, good hygiene programs, and good manufacturing practices during further processing, raw meat and meat products can be contaminated with pathogenic *E. coli* and *Salmonella* from fecal to hide to carcass contamination. These surface contaminants may progress through the food supply chain, especially pervasive in ground meat products. The residence of pathogens in undercooked meat is a serious food safety concern and carries the risk of resulting in a food-borne outbreak. Food processors of ready-to-eat (RTE) products containing beef or a beef product as an ingredient control *E. coli* O157:H7 by achieving a 5-log reduction. However, the concern is that certain strains are able to survive both the thermal interventions used in beef processing and subsequent cooking by the consumer.

Thermal processing is the application of heat to inactivate vegetative cells and is one main way of reducing pathogens present in a food product. Current cooking guidelines stipulate cooking ground beef products to 71°C and cooking poultry products to 74°C. However the variation in survival between heat-resistant and heat-sensitive organisms (Mercer et al., 2015) poses a risk to both the meat processing industry and the consumer. The thermal process lethal to the bulk of *E. coli* and *Salmonella* are withstood by heat-resistant strains (Dlusskaya et al., 2011; Mercer et al., 2015). In order to ensure appropriate reduction of pathogenic *E. coli* during

thermal processing further study is required. The heat resistance of *E. coli* depends on the characteristics of the food matrix including salt, fat, and water activity. Thermal stress induces shifts in the structure of membrane and cytoplasm, and deforms proteins, including ribosome and DNA, to cause cell death (Tsuchido et al., 1985; Mackey et al., 1991; Mohacsi-Farkas et al., 1999; Lee and Kaletunc, 2002). Hence, the process of cooking induces cell death and is traditionally effective in controlling bacteria survival. Heat resistant mechanisms of cells can accommodate and resist this heat stress through gene regulation of heat response in order to refold misfolded proteins as well as to stabilize membrane fluidity and accumulate compatible solutes. The survival of bacteria is dependent on the ability of the cell to retain membrane and protein function during thermal processing.

However, the threat to food safety increases if the pathogens present are in a food matrix that increases heat tolerance or are heat-resistant. While a number of studies have assessed the survival of *E. coli* and *Salmonella* in meat products, the potential influence of seasoning binder (containing NaCl) has not been investigated. Similarly, a significant amount of studies have examined the inactivation of pathogens in meat products the survival of heat-resistant *E. coli* and *Salmonella* in meat products has not yet been fully explored.

1.1 The meat industry

1.1.1 Overview of the Canadian beef industry

The beef industry is of economic and cultural relevance to Canada with 2.6 million head of cattle being slaughtered at federally-regulated establishments per year and $1.9 billion of beef products exported to 65 countries (Agriculture and Agri-Food Canada, 2017a). In 2015, Canadian farms and ranches raised 11.92 million cattle and calves. Canadian beef exports

totalled 322 thousand tonnes and were valued at $2.23 billion. The United States dominates most of the Canadian beef export market (71%; Agriculture and Agri-Food Canada, 2017a)

To improve meat safety it is important to effectively remove or inactivate bacterial contamination through interventions. The beef industry has multiple potential interventions that may be used on either the hide or the carcass. As hides of cattle are the primary source of carcass contamination, reducing the microbial load of the hide should result in reduced contamination of the hide off carcass (Villarreal-Silva et al., 2016). These hide-on interventions include chemical dehairing and cetylpyridinium chloride treatment, antimicrobial applications, on-line hide washing cabinets, and ozonated and electrolyzed oxidizing waters. The next stage of interventions is carcass interventions that decrease the level of pathogens on the surface of carcasses. These carcass interventions include steam vacuuming, organic acids and hot water, steam pasteurization and peroxyacetic acid (Ave et al., 2003; Gill and Bryant, 1997; Park et al., 2005). A multiple intervention approach is the most effective way to control contamination of a carcass with pathogens. The last step in the harvest of meat where interventions can be applied is on the primal cuts and trim. Interventions are used on trim help to minimize the risk of pathogens in ground products as trim is the precursor material for ground beef. It is well established that ground meat products are a source of pathogens (Laufer et al., 2015; Pollari et al., 2017).

Ground beef available to Canadian consumers varies by fat content. Labelling regulations stipulate that extra lean ground beef will not contain more than 10% fat, lean ground beef will not contain more than 17% fat, medium ground beef will not contain more than 23% fat, and regular ground beef will not contain more than 30% fat. It is important to consider fat content when working with ground beef, as it influences lethality (Ahmed et al., 1995).

In addition to *Escherichia* and *Salmonella*, genomic work identifying microbial genera on meat after storage included *Aeoromonas*, *Acinetobacter*, *Achromabacter*, *Alcaligenes*, *Alteromonas*, *Arthrobacter*, *Bacillus*, *Bradyhizobium*, *Brochothrix*, *Carnobacterium*, *Chromobacterium*, *Citrobacter*, *Clostridium*, *Enterobacter*, *Klebsiella*, *Kluyvera*, *Kocuria*, *Kurthia*, *Lactobacillus*, *Leuconostoc*, *Limnobacter*, *Listeria*, *Microbacterium*, *Micrococcus*, *Moraxella*, *Paenibacillus*, *Pantoea*, *Photobacterium*, *Proteus*, *Providencia*, *Pseudomonas*, *Psychrobacter*, *Rahnella*, *Ralstonia*, *Rudaea*, *Serratia*, *Shewanella*, *Staphylococcus*, *Stenotrophomonas*, *Streptococcus*, *Weissella*, and *Yersinia* (Doulgeraki et al., 2012). Beef, especially ground beef, is favorable for bacterial growth as it has a high-water activity with a surplus of nutrients and vitamins. In addition to being an ideal growth medium, retail beef is often subject to higher than ideal storage temperatures. The surface temperature of retail cuts can be as high as 10°C (Greer et al., 1994). This temperature abuse can result in proliferation of spoilage and pathogenic bacteria.

In 2012, the *Escherichia coli* O157:H7 outbreak associated with meat from XL Foods Inc. resulted in 1.8 million kilograms of beef being recalled (CBC, 2015). The recall was linked to raw beef trimmings that tested positive for the pathogenic *E. coli* O157:H7. This was confirmed by the United States Department of Agriculture's Food Safety and Inspection Service (USDA FSIS) that tested the beef as it was to be exported to the United States. The contaminated beef was linked to 18 illnesses. However, the XL Foods incident had a far greater impact, both financially and politically. At the time, XL Foods Inc. processing facility located in Brooks, AB represented 30% of Canada’s beef processing capabilities. Furthermore, multiple countries even halted the import of Canadian beef. An independent review of the incident cited the responsibility for the failure was on both the processor and the CFIA (CFIA, 2013). After the

review, one commitment made by the Canadian Government was to strengthen prevention strategies and regulatory oversight.

In February of 2017, CFIA approved the use of irradiation on fresh and frozen raw ground beef to reduce the microbial load. The Food and Drug Regulations define irradiation as treatment with ionizing radiation. Ionizing radiation treatment is considered as gamma-radiation from a Cobalt-60 or Cesium-137 source, X-rays generated form a machine source operated at or below an energy level of 5 MeV, and electrons generated from a machine source operated at or below an energy level of 10 MeV (CFIA, 2017). This approval came following the review of the 2012 XL Foods Inc. recall and as part of the Canadian Government's commitment to prevention strategies. However, the lack of processing facilities in Canada will limit availability of irradiated ground beef in Canada.

1.1.2 Overview of the Canadian poultry industry

In 2016, the Canadian poultry industry produced products worth $4.3 billion (Agriculture and Agri-Food Canada, 2017b). In 2016 there were 2,817 regulated chicken producers and 551 registered turkey producers. Of that, 70% of chicken producers are located in Ontario and Quebec. As of 2016, there were 40 federally inspected chicken slaughter plants in Canada, with the majority being in British Columbia, Ontario, and Quebec. Poultry exports (meat and byproducts; fresh, chilled, and frozen) totalled 165 million kilograms and were valued at $522 million (Agriculture and Agri-Food Canada, 2017b). These products were exported to 67 countries with largest foreign markets being the United States, Taiwan, Philippines, and Cuba.

The Canadian poultry industry commonly utilizes interventions to reduce the prevalence of pathogenic *E. coli*, *Salmonella* spp., and *Campylobacter* spp. Similar to the beef industry, the poultry industry has multiple interventions that may be implemented at pre-harvest or post-

harvest. Pre-harvest interventions are aimed at reducing likelihood of the presence of foodborne pathogens in birds on the farm. These strategies include good management practices and feed withdrawal. Feed withdrawal before catching contributes to reduced likelihood of carcass contamination. Post-harvest interventions are implemented to manage the risk of carcass contamination by reducing the microbial load and may be divided into slaughter and carcass interventions. Slaughter interventions include scalding, defeathering, and evisceration standards. Carcass interventions are in place to decontaminate carcasses and reduce the microbial load. These carcass interventions include carcass washes, hot water and steam treatments, chlorine carcass chilling, cetylpyridinium chloride application, electrolyzed oxidizing and ozonated water treatments and organic acid washes. Similar to the beef industry, multiple hurdle interventions are the most effective way to control for pathogens. As of 2017, CFIA has not approved the use of irradiation on poultry products to control for pathogens.

Canadian surveillance reports that *Salmonella* Heidelberg is the most frequently isolated serovar from Canadian chicken and turkey products (Public Health Agency of Canada, 2009).

1.2 Organisms of concern

1.2.1 Pathogenic *Escherichia coli*

E. coli are gram negative, non-spore forming, non-fastidious, motile, rod shaped bacteria that are facultative anaerobes and belong to the family *Enterobacteriaceae*. While most *E. coli* strains are non-pathogenic, the pathogenic strains are classified into pathotypes, six of which are associated with foodborne illness. The CDC (2017) characterizes these as Shiga toxin-producing *E. coli* (STEC), enterotoxigenic *E. coli* (ETEC), enteropathogenic *E. coli* (EPEC), enteroaggregative *E. coli* (EAEC), enteroinvasive *E. coli* (EIEC), and diffusively adherent *E. coli* (DAEC). The differentiation among the pathotypes is the presence or absence of specific

virulence factors. Each pathotype may contain various serotypes as many *E. coli* strains have common virulence factors. Serotyping is classifying *E. coli* based on reactivity of surface antigens to antibodies.

Symptoms of STEC infections include severe stomach cramps, bloody diarrhea, and potentially a fever. STEC infection symptoms require an incubation period usually occur 3-4 days after consuming contaminated food. A complication associated with STEC infections is hemolytic uremic syndrome (HUS) that is potentially life-threatening form of kidney failure. The most at-risk populations are young, elderly, and immunocompromised but all populations are still susceptible to infection.

Ruminants, such as cattle, are the reservoir for pathogenic and non-pathogenic *E. coli* (Elder et al., 2000). Subsequently, beef products are a major potential source for pathogens and the consumption of beef linked to foodborne illness. In 1994, the Food Safety and Inspection Service (FSIS) of the United States Department of Agriculture (USDA) declared *E. coli* O157:H7 an adulterant in raw ground beef. This decision came after the landmark Jack in the Box outbreak. This outbreak linked to undercooked burger patties resulted in more than 500 confirmed cases of *E. coli* O157:H7 infections and 4 deaths. The source of the contaminated beef trim was attributed to five American primary processing facilities and one Canadian facility. (CDC, 1993)

In 2012 the USDA added 6 additional STEC serogroups to their list of adulterants in raw ground beef; *E. coli* O26, *E. coli* O45, *E. coli* O103, *E. coli* O111, *E. coli* O121 and *E. coli* O145. It is important to note that in Canada there are no regulations that determine *E. coli* O157:H7 and the other six to be adulterants in raw ground beef. However, that sampling is

required by Canadian processors in order to meet requirements to export beef products to the USA.

1.2.2 *Salmonella*

Salmonella are a gram negative, non-spore forming, motile, rod-shaped bacterium that are facultative anaerobes. *Salmonella* is a member of the *Enterobacteriaceae* family. The *Salmonella* genus is divided into 2 species, *Salmonella enterica* and *Salmonella bongori*. *Salmonella enterica* is separated into subspecies. The only subspecies associated with foodborne illness is *S. enterica* subspecies *enterica*. Of this subspecies, there are 1586 known serovars (Issenhuth-Jeanjean et al., 2014). Domesticated meat animals can be carriers of *Salmonella* that cause illness in humans. *Salmonella* serovars can be host adapted and persist in domestic animal populations (Kingsley and Bäumler, 2000). However, non-host adapted strains are the hazard in food products.

Symptoms of *Salmonella* infections include fever, chills, diarrhea, abdominal pains, headache, nausea, and vomiting. Salmonellosis symptoms usually occur 6-48 h after consuming contaminated food. Typically affected food is contaminated through the fecal-oral route. While anyone can contract salmonellosis the most at-risk populations are the young, elderly, and immunocompromised. The assessment from outbreaks suggests that fewer than 10 cells may be enough to cause symptomatic illness (Kapperud et al., 1990; Lehmacher et al., 1995; Abe et al., 2004). The other type of *Salmonella* infection is Typhoid fever. Typhoid fever is caused by *Salmonella* Typhi, requires a higher infective dose and longer incubation period but has a higher mortality than salmonellosis (Hammock, 2012).

Typically, *Salmonella* causes salmonellosis when food products are contaminated or abused in a manner that allows growth of the organism. *Salmonella* are typically associated with

raw poultry. In Canada, *Salmonella* has been identified as causing 5% of foodborne illnesses but causes 24% of hospitalizations and 17% of deaths (Health Canada, 2016). Raw meat can be contaminated with *Salmonella* during the slaughter process if the animal is a carrier. While *Salmonella* is more commonly associate with poultry products it is still a hazard in ground beef. The prevalence of *Salmonella* in ground beef was reported to be 4.2% with contamination from the hide and lymph nodes (Bosilevac et al., 2009; Koohmaraie et al., 2012). While out of the scope of this thesis it is important to mention that *Salmonella* outbreaks have been linked with a myriad of products other than meat products including egg shells, sprouts, pistachios, cucumber, peanut butter, mango, and cantaloupe. *Salmonella* Typhi is not directly associated with food products but instead is transmitted by food production workers that are human carriers of the disease (Connor and Schawrtz, 2005).

Currently, Health Canada recommends cooking poultry products to an internal temperature of 74°C (Health Canada, 2014). However, reaching an internal temperature of 74°C may not always be achieved by the consumer or even be sufficient to achieve the required thermal destruction. Recently *Salmonella* has been the cause of recalls of frozen raw breaded chicken products (PHAC, 2017). These frozen raw breaded chicken products must be fully cooked prior to consumption. However, as they are lightly breaded and par-fried they have the golden-brown color associated with cooked products that the public may mistake as fully cooked. During a 2003 outbreak associated with frozen processed chicken nuggets and chicken strips, one third of individuals affected considered these products to be precooked and thought the products only required reheating (MacDougall et al., 2004). Additionally, one quarter of cases responded that they used a microwave oven for heating the products. Microwave cooking is considered an inappropriate cooking method as the uneven heating makes it difficult to ensure

that a sufficient internal temperature is reached throughout the product. Since 2015, there have been 3 national outbreak investigations linked with frozen raw breaded chicken products. The latest national outbreak (PHAC, 2017) linked with frozen, raw breaded chicken products affected 18 individuals, and caused 6 people to be hospitalized, and so far, has resulted in one death. There have been similar recalls of uncooked stuffed chicken products but there have been no reported illnesses from consumption.

The most notable thermotolerant *Salmonella* is *S. enterica* subsp. *enterica* serovar Senftenberg. This serovar was originally derived from dried egg powder in the 1940s and initially published as strain 775W (Liu et al., 1968; Ng et al., 1969). There were multiple cases of salmonellosis associated with consumption of dried egg powder thought to be the result of the presence of *S. enterica* serovar Senftenberg (Solowey et al., 1947; McCoy, 1975).

1.3 Thermal destruction of pathogens

1.3.1 Thermal processing

Thermal processing has a long-standing history to control pathogens in the food supply. Thermal processing, or the application of heat, inactivates or disrupts the cellular membrane, proteins, and ribosomes of a cell (Tsuchido et al., 1985; Mackey et al., 1991; Mohácsi-Farkas et al., 1999; Lee and Kaletunc, 2002).

The classical understanding of the thermal inactivation of *E. coli* and *Salmonella* has been a log linear decline in survivors based on exposure time to thermal processing. For the last several decades there have been multiple models developed to determine thermal treatment times for pathogens in different food matrices. This is due to deviations from the traditional log linear model, including sigmoidal survivor curves, and shouldering or tailing of the survivor curves. Some alternate models include: log linear biphasic (Cerf, 1977), log linear with (Geeraerd et al.,

2000), log linear with shoulder (Geeraerd et al., 2000), Weibull (Mafart et al., 2001), and double Weibull (Coroller et al., 2006). Thermal treatment in combination with a secondary treatment, such as with addition of an antimicrobial, affects the shape of the survivor curve and thus changes the appropriate predictive model (Juneja et al., 2013). It is important for food processors to take into account the model when considering the thermal inactivation of their product to ensure sufficient reduction of pathogens.

The result of thermal inactivation is expressed as a *D*-value, the time required at a given temperature to cause a 1-log reduction of the treated cells. *D*-values are organism specific and vary based on food matrix. Substantial research has been done related to characterizing the thermal inactivation of *Salmonella* in ground beef (Smith et al., 2001; Table 1) and chicken products (Murphy et al., 2002; Juneja et al., 2001; Table 2). For *Salmonella* Typhimurium Smith et al. (2001) reported a higher *D*-value in ground beef at a higher fat content (19.1% compared to 4.8%) at 55°C. The authors also reported higher *D*-values of an 8-serotype cocktail of *Salmonella* grown to stationary phase compared to the same cocktail used while in the log phase of growth. Additionally, it was noted that freezing ground beef before cooking decreased the *D*-value of the *Salmonella* cocktail inoculum.

The CFIA requires 7-log_{10} reduction in *Salmonella* spp. in cooked ready-to-eat (RTE) products containing poultry (CFIA, 2016). For meat products not containing poultry, CFIA only require a 6.5-log_{10} reduction in *Salmonella* spp. Manufacturers must be able to validate that their process is capable of sufficient thermal destruction of the target organism. However for heated, non-ready-to-eat products (NRTE) manufacturers are not required to verify the same thermal reduction. Operators instead must ensure that their product is labelled in a way to inform consumers that the product is not cooked so consumers do not mistake the product as RTE. The

cooking instructions should ensure the same 7-log_{10} reduction in *Salmonella* spp. when instructions are followed by the consumer. These heated NRTE products include frozen raw breaded chicken products including chicken nuggets and chicken tenders. Raw breaded chicken products routinely have a thermal processing step or par-cook to set their breading or batter prior to freezing and packaging. This may mistakenly give the consumer the impression that the product is cooked or only requires reheating. Additionally, the CFIA stipulates that the operator must ensure the instructions use equipment comparable to the equipment available to the consumer.

Table 1: D-values of *Salmonella* in ground beef. Adapted from Smith et al. (2001).

Serotype of *Salmonella*	Medium	D-value (min) at Temp (°C)			
		55	58	61	64
Senftenberg	Ground beef (19.1% fat)	ND	21.8	3.38	0.92
Typhimurium DT104 (10127)	Ground beef (19.1% fat)	21.98	2.63	0.65	0.16
Typhimurium DT104 (10127)	Ground beef (4.8% fat)	9.08	2.26	0.57	0.15
Typhimurium DT104 (10601)	Ground beef (4.8% fat)	10.6	2.15	0.41	0.07
Typhimurium DT104 (01071)	Ground beef (4.8% fat)	10.27	2.06	0.43	0.14
8-serotype cocktail, log phase	Ground beef (19.1% fat)	16.3	2.72	0.44	0.15
8-serotype cocktail, stationary phase	Ground beef (19.1% fat)	18.7	3.39	0.57	0.20
8-serotype cocktail, log phase frozen	Ground beef (19.1% fat)	9.85	1.43	0.29	0.14
8-serotype cocktail, stationary phase	Ground beef (19.1% fat)	12.5	2.36	0.28	0.20

ND=Not Determined

Table 2: D-values of *Salmonella* in chicken and beef products. Adapted from Murphy et al. (2002) and Juneja et al. (2001).

Serotype	Medium	D-value (min) at Temp (°C)						
		55	57.5	60	62.5	65	67.5	70
8-serotype cocktail	Ground chicken (2% fat)	ND	ND	4.83	1.14	0.415	ND	ND
8-serotype cocktail	Ground chicken (6.3% fat)	ND	ND	4.68	1.16	0.314	ND	ND
8-serotype cocktail	Ground chicken (9% fat)	ND	ND	5.40	1.16	0.529	ND	ND
8-serotype cocktail	Ground chicken (12% fat)	ND	ND	5.50	1.30	0.509	ND	ND
6-serotype cocktail	Chicken patties (5.36% fat)	26.7	14.55	8.09	3.98	1.39	0.61	0.32
6-serotype cocktail	Chicken tenders (21.43% fat)	22.37	9.92	8.50	4.55	1.25	0.38	0.32
6-serotype cocktail	Beef patties (18.56% fat)	9.09	7.70	4.80	2.40	0.97	0.57	0.25
6-serotype cocktail	Beef-turkey patties (20.11% fat)	20.58	12.89	4.42	2.04	1.03	0.71	0.37

ND=Not Determined

1.3.2 Mechanisms of heat resistance

As thermal processing is often used to reduce pathogen numbers in the foods, it is important to understand that there are a multitude of bacterial mechanisms involved in the ability of a cell to resist heat stress and survive thermal treatment. Heat-resistant *Enterobacteriaceae* are a recent challenge for the meat industry and pose a food safety hazard. Heat resistance in *Enterobacteriaceae* is the result of alternative sigma factors (Dodd and Aldsworth, 2002: Noor, 2015), specialized heat shock proteins (Arsene et al., 2000), and acquisition of compatible solutes (Hengge-Aronis et al., 1991; Li and Ganzle, 2016; Pleitner et at., 2012).

The cellular membrane and imbedded proteins are the initial defence of the cell to environmental stress. Extracellular polysaccharides such as colonic acid confer heat tolerance by encasing the cell in a polysaccharide matrix (Whitfield and Valvano, 1993; Mao et al., 2001). The presence of divalent cations stabilizes a lipopolysaccharide layer to prevent the mobility of hydrophobic molecules (Hitchener and Egan, 1997; Vaara, 1992; Hauben et al., 1998).

The fluidity of the cellular membrane is an important factor in heat resistance as it influences membrane function (Zhang and Rock, 2008). An increase in temperature increases the fluidity of the cellular membrane usually resulting in disrupted activity. An increase in saturated fatty acid content of the membrane is linked to retained function at higher temperatures. Heat resistant strains had a higher proportion of cyclopropane fatty acids in the phospholipid membrane than heat sensitive strains (Chen and Gänzle, 2016). Additionally that an increase in cyclopropane fatty acid synthesis resulted in increased heat resistance in heat-sensitive and heat-resistant *E. coli* (Chen and Ganzle, 2016). The heat resistance of *E. coli* slowly adapted to higher temperatures was attributed to adaptation of membrane fluidity (Guyot et al., 2000).

The regulation of heat shock proteins is another key mechanism in heat resistance. Alternative sigma factors regulate cytoplasmic and periplasmic heat shock responses. The *rpoE* gene encodes for an alternative sigma factor σ^E (Raina et al., 1995) and *rpoS* encodes for an alternative sigma factor σ^S. The function of *rpoE* is to respond to general stress experienced by the cell. The regulation of *rpoS* responds to multiple stressors (osmotic, heat, nutrient deficiency, and oxidative; Dodd and Aldsworth, 2002). The response to heat is to promote gene expression of heat shock proteins including chaperones and proteases. These heat shock proteins are responsible for maintaining protein structure by supporting the structure of semi-denatured

proteins and preventing the aggregation of denatured proteins (Parsell and Lindquist, 1993; Landini et al., 2014; Lee et al., 2016).

1.3.3 Locus of heat resistance

Classification of heat resistance in strains is based on heat resistance phenotypes of highly heat resistant, moderately heat resistant, and heat sensitive. High heat-resistance was defined by Mercer et al. (2015) to be any strain that had a D_{60}-value greater than 6 min in broth. Moderate heat-resistant and heat-sensitive strains were any strain that had a D_{60}-value greater than 1 min in broth and less than 1 min, respectively. In particular one highly heat resistant strain, *E. coli* AW1.7, was isolated from a carcass after it had been washed at a federally inspected meat facility (Aslam et al., 2004). Mercer et al. (2015) sequenced a total of 28 strains from food and clinical sources, including 19 STEC, with differing degrees of heat resistance. Through comparative genomics the heat resistance of *E. coli* AW1.7 was ascribed to a 14-kb genomic island unique to phenotypically highly heat resistant strains. This region was denoted locus of heat resistance (LHR).

The LHR contains 16 open reading frames (ORF) with 6 being wholly unique to the heat resistance conferred by the LHR (Figure 1). The LHR is flanked by putative mobile elements. The LHR encodes several putative heat shock proteins, proteases, and transport proteins (Mercer et al., 2015). LHR positive-strains survive thermal interventions that are lethal to LHR-negative strains (Dlusskaya et al., 2011; R. G. Mercer et al., 2017; Mercer et al., 2015). Heat-resistance is not exclusive to non-pathogenic strains of *E. coli* as Ma and Chui (2017) detected the LHR in 3 of 613 clinical isolates.

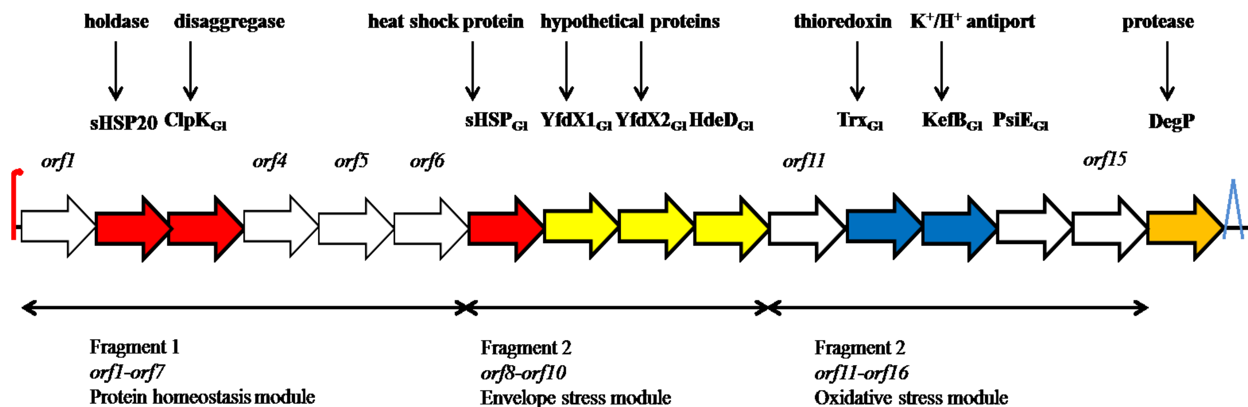

Figure 1: The genomic island (LHR) determined from *E. coli* AW1.7. Color is based on predicted function: heat shock proteins (red), hypothetical proteins (yellow), proteins related to oxidative stress (blue), and DegP (orange). Functions of numbered ORFs are not yet known. Schematic is adapted from Mercer et al. (2017) and based on previously published data (Lee et al., 2016; Mercer et al., 2015; Lee et al., 2015; Boll et al., 2017; Li., 2017).

To confirm that the LHR was responsible for providing heat resistance the full sequence of the LHR and partial fragments of the LHR were constructed on a plasmid and introduced to heat-sensitive strains (Mercer et al., 2015). A transgenic LHR-positive strain was created by introducing the plasmid containing the LHR to the LHR negative, heat sensitive derivative of *E. coli* AW1.7, *E. coli* AW1.7 ΔpHR1. The wild type strain and transgenic LHR-positive strain had comparable heat resistance and the LHR was confirmed to confer heat resistance (Mercer et al., 2015).

The LHR has been investigated by other research groups that proposed that the LHR be named "transmissible loci for protein quality control (TLPQC)." This is due to the other ORFs on the genetic material being homologous to proteins involved in protein homeostasis. Phylogenetic analysis revealed two TLPQCs present in the thermotolerant *S. enterica* serovar Senftenberg ATCC 43845. Nguyen et al. (2017) found that TLPQC1 and TLPQC 2 were present

on a single plasmid. The core genes of TLPQC1 are predicted to encode a phage-like transcriptional regulator, two heat shock proteins, *CLp* protease, cardiolipin synthase, ATP-dependant *FtsH* protease, two *YfdX* proteins, thioredoxin, K^+/H^+ antiporter , *PsiE* protein, a zinc-dependant protease, and a periplasmic serine protease (Nguyen et al., 2017; Lee et al., 2016). The TLPQC2 shares high similarity to the LHR. An additional third loci, TLPQC3, was described as the shortest and predicted to encode a heat shock protein, *Clp* protease, cardiolipin synthase, a zinc-dependant protease, and a periplasmic serine protease (Nguyen et al., 2017). For the purposes of this thesis the nomenclature of LHR will be maintained.

1.3.4 Heat resistance in a food matrix

As heat treatment and thermal processing is used in the food industry to control pathogens it is important to understand how different food matrixes play a role in the heat resistance and influence survival. Processors lack information that can be used for their products regarding *D*- and *z*-values of heat-resistant organisms. The differences in composition influence the thermal inactivation kinetics, especially in meat matrices (Juneja et al., 1998).

When referring to a meat matrices there are ground meats and intact cuts. As previously mentioned ground meats are more likely to be contaminated with pathogens and linked to recalls. It is generally understood that the interior of intact cuts of beef is sterile. However, pathogens can be introduced into muscle cuts through blade tenderization (Luchansky et al., 2013). *Salmonella* is more heat resistant in whole muscle than in ground beef (Orta-Ramirez et al., 2005).

The effect of salt on increasing heat resistance was demonstrated by Pleitner et al. (2012) when *E. coli* AW1.7 was exposed to osmotic stress. *E. coli* AW1.7 was grown in liquid culture at different NaCl concentrations (2-6%) to expose the cells to varying intensities of osmotic stress.

As the osmotic stress increased so did the heat resistance of *E. coli* AW1.7. This phenomenon was explained by accumulation of compatible solutes (Pleitner et al., 2012). Concentrations of NaCl between 2.7% and 4.7% in ground beef increased the heat resistance of *E. coli* O157:H7 at temperatures up to 62.5°C (Juneja et al., 2015). Similarly when exposed to 5% salt and stored at room temperature for 24 h prior to heating, *E. coli* O157:H7 had increased heat resistance at 55°C (Juneja et al., 2015). It is not known if the same effect would be observed if the culture was stored at refrigeration temperatures prior to thermal processing.

The effect of fat and salt on increasing heat resistance was reported by Liu et al. (2015) *E. coli* AW1.7 was inoculated into ground beef patties with 15% and 35% fat with 0% or 2% NaCl and cooked to an internal temperature of 63°C and 71°C. The greatest heat resistance for *E. coli* AW 1.7 was observed in the patties with high fat (35% fat) and 2% NaCl.

When considering thermal lethality it is important to consider product composition as it plays a significant role in thermal inactivation. This is especially true for meat products as they often have varying formulations differing in physical characteristics, salt content, and fat percentage.

1.3.5 Seasoning binder in food matrices

The meat industry includes seasoning and binder ingredients into their products to improve taste and quality. These products include salt (NaCl) and spices that acts as flavor enhancers. Non-meat proteins (wheat, whey, and soy) improve water binding, stabilize fats, and reduce product costs (Hsu and Sun., 2006; Andres et al., 2006). Toasted wheat crumb is considered binder filler. Seasonings and binders are important as they improve the sensory qualities of fried foods that have batter coatings. Batter coatings reduce moisture loss during drying and reduce oil absorption (Mohamed et al., 1998). Batters are classified into

interface/adhesion and puff/tempura (Loewe, 1990). Interface or adhesion batters rely on a breaded surface between the surface of the product being fried and the batter itself. Puff/tempura batters are composed of wheat flour, corn flour, and leavening agent as key components. Baking powder is a common leavening agent used. The variations in flours determine batter viscosity and contribute to the overall quality of fried foods. The inclusion of soy flour in puff/tempura batters increased batter viscosity that resulted in improved crispness and color and reduced oil absorption (Dogan et al., 2004).

Most research that has been done on seasoning binder in food matrices has focused on the functional characteristics and effect on food quality. Several papers have examined the effect of the addition of novel additives with antimicrobial properties to meat products to increase heat sensitivity of pathogens. The addition of trans-cinnamaldehyde increased the reduction of *E. coli* O157:H7 in cooked ground beef patties (Amalaradjou et al., 2010). Similarly, olive extracts and lemongrass essential oil both increased the reduction of *E. coli* O157:H7 in ground beef patties (Rounds et al., 2012). However, these novel additives are generally not practical for industrial use as they can impart distinct sensory characteristics (Nadarajah et al., 2005; Ntzimani et al., 2010). Little research has been done on common ingredients in meat products and the role they play in survival of pathogens.

1.4 Research objectives

This research aimed to test the hypothesis that seasoning binder increased survival of heat-resistant and heat-sensitive strains in meat products.

The specific objectives were to:

1) determine the thermal inactivation values of *E. coli* AW1.7 and *S. enterica* ATCC 43845 in ground beef;

2) investigate the range of survival of heat-resistant organisms in ground beef with different processing parameters; and

3) determine the inactivation of heat-resistant organisms in fully cooked breaded and frozen chicken nuggets.

2. Materials and Methods

2.1 Bacterial strains and culture conditions

Bacterial strains used in this research are listed in Table 3. Cultures were maintained as frozen (-80°C) stocks in LB broth with 50% glycerol. *E. coli* and *S. enterica* isolates were grown individually in Luria-Bertani broth (LB; Difco; Becton, Dickinson and Company, Sparks, MD). To maintain the plasmid in *E. coli* AW1.7 Δ pHR1, strains were grown in LB broth supplemented with 15 mg/L tetracycline-HCl. Bacterial cultures were incubated 18-22 h at 37°C to obtain an inoculum concentration of *ca* 10^7 CFU/mL. To prepare a cocktail of VTEC, equal volumes of all 5 strains were combined. Cells were not removed from supernatant prior to inoculation of meat.

2.2 Determination of *D*- and *z*-values in ground beef

2.2.1 Ground beef preparation

Fresh ground beef (24% fat) was obtained from a federally inspected meat processor. The ground beef was separated into portions of 200 g, vacuum packaged and held at -20°C until 1 d prior to use. Ground beef was thawed overnight at 4°C. Ground beef was inoculated with individual strains of *E. coli* AW1.7 or *S. enterica* ATCC 43845 by mixing 10 mL of an overnight culture with 200 g of ground beef. For treatments with seasoning binder, the seasoning binder [Glib Burger Seasoning and Binder (Griffith Foods, Toronto, ON) containing toasted wheat crumb and 17.5% NaCl; 5.6% w/w)] was added to the meat immediately after inoculation. The water activity of the ground beef and ground beef with seasoning binder was measured using a water activity meter (Aqualab PRE; Decagon Devices, WA, USA). The uninoculated ground beef had total aerobic plate counts and coliform counts below 3000 CFU/g and 100 CFU/g, respectively.

Table 3: Bacterial strains used in this work

Organism	Description	Reference (if any)
Escherichia coli FUA 1675	LHR-positive; Isolated from fully cooked breaded chicken product from a processing facility	
Escherichia coli AW1.7	LHR-positive wild type food isolate	Dlusskaya et al., 2011
Escherichia coli AW1.7 ΔpHR1	LHR-negative, heat-sensitive derivative of AW1.7	Pleitner et al., 2012
Escherichia coli AW1.7 ΔpHR1(pLHR)	Transgenic LHR-positive derivative	Mercer et al., 2015
Escherichia coli O157:H7 FUA 1305	VTEC cocktail	Liu et al., 2012
Escherichia coli O145:NM FUA 1307	VTEC cocktail	Liu et al., 2012
Escherichia coli O26:HM FUA 1308	VTEC cocktail	Liu et al., 2012
Escherichia coli O121:H19 FUA 1312	VTEC cocktail	Liu et al., 2012
Escherichia coli O145:NM FUA 1674	VTEC cocktail; PARC 449	Garcia-Hernandez et al., 2015
Salmonella enterica subsp. *enterica* serovar Typhimurium ATCC 13311	LHR-negative	ATCC
Salmonella enterica subsp. *enterica* serovar Senftenberg ATCC 43845	LHR-positive	ATCC

2.1.2 Thermal Treatment

The method to determine *D*- and *z*-values was adapted from Murphy et al. (2004). Inoculated ground beef (10 g) was vacuum packaged (Model C200; Multivac, Kansas City, MO) in vacuum bags, rolled until evenly flattened to *ca* 1 mm thick and placed in a heated circulating water bath (Precision Scientific CO, Chicago, IL) at a set temperature. Samples were removed from the water bath at set times and immediately immersed in ice water for 20 min. Sample bags were opened aseptically and 40 mL of sterile 0.1% peptone water was added to each sample to

achieve a 1:4 dilution. The samples were stomached for 2 min and serial 10-fold dilutions were prepared and plated onto LB agar (Difco). Plates were incubated at 24 h at 37°C prior to enumeration.

2.3 Heat treatment in ground beef

2.3.1 Burger preparation

2.3.1.1 Effect of seasoning binder after 48 h storage

To evaluate the effect of seasoning binder (containing NaCl) after 48 h storage at refrigeration conditions on survival heat-resistant and heat-sensitive organisms were inoculated into beef patties with and without 5.6% added seasoning binder. Fresh ground beef (24%) was prepared as described above. Ground beef was thawed overnight at 4°C. Ground beef was inoculated with individual strains of *E. coli* or *S. enterica* by mixing 10 mL of overnight cultures with 200 g of ground beef. The ground beef was massaged by hand in a sterile bag for 2 min. The ground beef (200 g) was formed into a patty with a diameter of 12 cm with a single hamburger press (Weston Brand Pragotrade, Strongsville, OH, USA). The patties were placed on Styrofoam trays, wrapped with oxygen permeable plastic wrap, and stored at 4°C for 48 h. After storage and prior to cooking, a 20 g sample was removed for enumeration of initial inoculum. Patties were cooked as described below.

2.3.1.2 Effect of end point core temperatures

To evaluate the effect of different end point core temperatures beef patties inoculated with *E. coli* AW1.7 were cooked to an internal temperature of 63, 71, or 74°C. Fresh ground beef (24%) was prepared as described above. Ground beef was thawed overnight at 4°C. Ground beef was inoculated *E. coli* AW1.7 by mixing 10 mL of overnight cultures with 200 g of ground beef.

The ground beef was massaged by hand in a sterile bag for 2 min. A 20 g sample was removed for enumeration of initial inoculum. The remaining ground beef (180 g) was formed into a patty with a diameter of 12 cm single a single hamburger press. Patties were cooked immediately as described below.

2.3.1.3 Effect of seasoning binder on survival of VTEC

To evaluate the effect of seasoning binder (containing NaCl) on survival of VTEC after cooking of meat a 5-strain VTEC cocktail were inoculated into beef patties with 0%, 5.6% and 16.8% added seasoning binder. Fresh ground beef (24%) was prepared as described above. Ground beef was thawed overnight at 4°C. Ground beef was inoculated with the VTEC cocktail by mixing 10 mL of the cocktail with 200 g of ground beef. For treatments with seasoning binder, the seasoning binder was added after inoculation. The ground beef was massaged by hand in a sterile bag for 2 min. The inoculated meat mixture was stored at 4°C for 24 h. After storage a 20 g sample was removed for enumeration of initial inoculum. The remaining ground beef (180 g) was formed into a patty with a diameter of 12 cm single a single hamburger press. Patties were then cooked immediately as described below.

2.3.2 Cooking procedures and determination of survival in ground beef patties

Burgers for each experiment were prepared as above. To monitor internal temperature of the ground beef patties during cooking, 4 thermistor probes (Tinytag Thermistor Probes; Gemini Data Loggers, Chichester, West Sussex, UK) connected to 4 thermocouples (Tinytag TV-4020; Gemini Data Loggers, Chichester, West Sussex, UK) were inserted into the patty, see Figure 2.

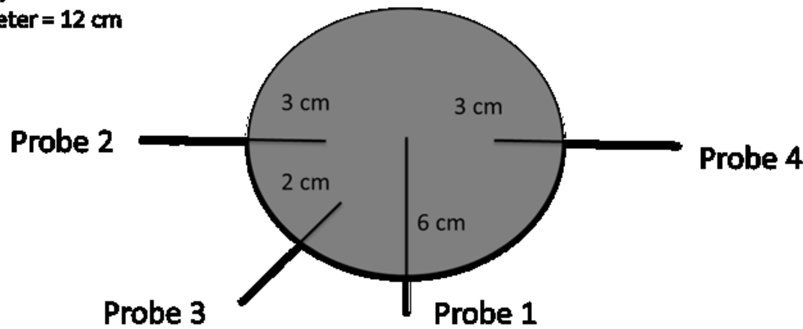

Figure 2: Thermistor probe placement in ground beef patty during cooking. Probe 1 reflects geometric center of the patty. Probe 2 reflects the left hand side of the patty. Probe 3 reflects the corner of the patty. Probe 4 reflects the right hand side of the patty.

For the experiment where patties were cooked immediately, the method of Mercer et al. (2017) was used. For all experiments, a clamshell grill (Cuisinart, Woodbridge, ON) preheated to 177°C was used for cooking patties. Patties were cooked until all probes reached 71°C unless otherwise stated.

After cooking, the patty was immediately placed in a sterile Whirlpak bag with 200 mL peptone water (Difco) that had been held on ice until burgers were processed (<20 min). Samples were stomached for 2 min, serially diluted in sterile 0.1% peptone water and plated onto LB agar. Plates were incubated at 37°C for 24 h prior to enumeration.

TinyTag Explorer software v4.7 (Gemini Data Loggers, Chichester, West Sussex, UK) was used to set up data loggers and view recorded data. Data loggers were configured to take a measurement every 5 sec and measure the temperature at the end of each interval.

2.4 Heat treatment in ground chicken

2.4.1 Ground chicken preparation

To prepare aseptic ground chicken breast the method of Liu et al. (2014) was used. Raw chicken breasts with fillets were purchased from a local grocery store (Edmonton, AB) and processed immediately. Chicken breasts were removed from packaging and immersed in hydrogen peroxide for 3 min, removed and air dried. The breasts were then submerged in 98% ethanol for 1 min and flamed. The discolored surface tissue was removed from the chicken breasts with a sterile surgical blade. The chicken breasts were ground in a food processor (Cuisinart, Woodbridge, ON) disinfected with 70% ethanol to achieve a coarse grind.

The ground chicken breast was separated into portions of 81 g, vacuum packaged and held at -20°C until 1 d prior to use. Initial total aerobic count of uninoculated ground chicken breast were below detection limit [<2 log (CFU/g)].

2.4.2 Cooking procedures and determination of reduction in chicken nuggets

To prepare chicken nuggets, the method was adapted from Gremmelspacher (2016). Previously ground chicken breast was thawed overnight at 4°C. The chicken nugget formulation was derived from a formulation recommended by Griffith Foods (Toronto, ON), listed in Table 2. Chicken nugget seasoning binder (2.5 g) was hydrated with sterile water (7.5 mL) in a sterile bag for 2 min. The ground chicken breast (81 g) was added and massaged by hand for 1.5 min. The canola oil (9 mL) was added and massaged by hand for 1 min. Chicken nugget batter was inoculated with individual strains of *E. coli* or *S. enterica* mixing 4 mL of overnight cultures into the meat mixture. Inoculated chicken nugget batter (65 g) was portioned into a petri dish, shaped into patty, sealed with parafilm, and stored overnight at -20°C.

The frozen chicken nugget was cut into individual nuggets at *ca* 16 g each. Each nugget was lightly coated with chicken Nugget Predust (Griffith Foods, Toronto, ON). The nuggets were dipped in batter [1 part Krusto Batter 4352 No Egg/Dairy (Griffith Foods, Toronto, ON): 1.5 parts sterile water]. Nuggets were removed from the batter and excess batter was allowed to drip off before nuggets were coated in breading [KB 8800 Breading (Griffith Foods, Toronto, ON)]. Ingredients of the seasoning, batter, and breading are listed in Table 3. Chicken nuggets were breaded to *ca* 25 g each. For all experiments, a deep-fryer (T-Fal, Toronto, ON) with filled with canola oil was used for cooking nuggets. The nuggets were par-cooked for 30 sec at 190°C. Nuggets were left to air-cool for 5 min, packaged in polypropylene jars, and stored overnight at -20°C. Ground chicken breast and chicken nugget batter samples were taken and water activity measured as described above.

Table 4: Composition of chicken nuggets

Ingredient	Percentage (%)
Ground chicken breast	81
Water	7.5
Chicken Nugget Seasoning Binder	2.5
Canola oil	9

Frozen nuggets were cooked in canola oil at 180°C. To monitor internal temperature of the nugget during cooking, 1 thermistor probe (attached to Tinytag View 2 data loggers) was inserted into the geometric center. Nuggets were cooked until the probe reached 74°C.

For enumeration, the nugget was immediately placed in a sterile Whirlpak bag with 100 mL refrigerated peptone water which was held on ice until nuggets were processed. Samples

were stomached for 2 min, serially diluted in sterile 0.1% peptone water and plated onto LB agar. Plates were incubated at 37°C for 24 h prior to enumeration.

Table 5: Composition of ingredients used in preparation of meat products

Component	Ingredients
Glib Burger Seasoning & Binder	Toasted wheat crumbs, salt, spices (with mustard), autolyzed yeast extract, high monounsaturated vegetable oil (canola &/or soybean &/or sunflower seed) (MFG aid)
Chicken Nugget Seasoning Binder	Salt, soy, protein isolate, flavour, vegetable oil (canola oil &/or sunflower)
Chicken Nugget Predust	Wheat flour, toasted wheat crumbs, wheat gluten, salt, spices, vegetable oil (canola &/or sunflower) (mfg aid), yeast extract, skim milk powder, garlic powder, silicon dioxide (mfg aid), dextrose, citric acid
Krusto Batter 4352 No Egg/Dairy	Modified corn starch, wheat flour, yellow corn flour, salt, baking powder, modified palm oil, defatted soy flour, guar gum
KB 8800 Breading	Toasted wheat crumbs with spices [wheat flour, baking powder, dextrose, salt, monoglycerides, yeast, spices, dough conditioners (ascorbic acid, L-cysteine monohydrochloride)]

TinyTag Explorer software v4.7 (Gemini Data Loggers, Chichester, West Sussex, UK) was used to set up data loggers and view recorded data. Data loggers were configured to take a measurement every 5 sec and measure the temperature at the end of each interval.

2.5 Statistical analysis

2.5.1 *D*-, *z*-, and F-values

All experiments were replicated three times (with one exception for *S. enterica* ATCC 43845 with seasoning binder where duplicates were completed). All microbiological counts were converted to $\log_{10}$ (CFU/g) prior to data analysis.

Data were entered into a Microsoft Excel spreadsheet and *D*- and *z*-values were calculated. The log_{10} of the number of surviving *E. coli* AW1.7 and *S. enterica* ATCC 43845 in ground beef after each heat treatment were plotted against thermal treatment time. The *D*-value at each temperature was calculated from the linear regression model for the log_{10} of recovered cells and heating time. The *D*-value is the negative inverse slope of recovered cells plotted against heating time, as expressed by equation (1).

(1) $\log_{10}(N) = \log_{10}(N_0) - t/D$

N = number of surviving cells at time t

N_0 = number of surviving cells at time 0

t = heating time

Significant differences between means of *D*-values was determined by using an unpaired T-test assuming equal variance ($p<0.05$).

The *z*-values for *E. coli* and *S. enterica* were determined by the linear regression of the log_{10} of *D*-values and corresponding temperature. The *z*-value is the negative inverse of *D*-values plotted against treatment temperature, as expressed by equation (2)

(2) $\log_{10}(D) = \log_{10}(D_0) - T/z$

where D = D-value at temperature T; T = Treatment temperature

The process lethality as expressed by equation (3). The core temperatures are the temperatures from the lowest temperature probe, as in the coldest part of the patty.

(3) $F = t_1 \times 10^{-(T_{ref}-T1)/z} + t_2 \times 10^{-(T_{ref}-T2)/z} + \ldots$

where F = total process lethality; t = time; T = core temperature at time t; T_{ref} = reference temperature; z = *z*-value

The hypothetical log reduction of the process is expressed by equation (4).

(4) Log Reduction of Process = F/D_{ref}

where F = calculated total process lethality; D_{ref} = D-value at temperature T_{ref}

2.5.2 Heat treatment in meat

All cooking experiments were replicated three times. For experiments with nuggets, four nuggets were prepared for each replication, one was randomly selected for precook enumeration and three were cooked. All microbiological counts were converted to $\log_{10}$ (CFU/g) prior to data analysis. Data were subjected to ANOVA and mean separation with Tukey's post-hoc test.

3. Results

3.1 Effect of seasoning binder on *D*- values of *E. coli* AW1.7 and *S. enterica* ATCC 43845 in ground beef

To determine the effect of seasoning binder on *D*-values of *E. coli* AW1.7 and *S. enterica* ATCC 43845 in ground beef, strains were inoculated into ground beef and heated at 55 to 70°C. Initial counts of total aerobic and coliform bacteria on uninoculated ground beef were less than 3000 and 50 CFU/g, respectively. Table 3 shows *D*-values for *E. coli* AW1.7 *and S. enterica* ATCC 43845 heated in ground beef with 24% fat, with or without 5.6% seasoning binder. No obvious shoulders or concaves were observed in the survivor curves (Figure 3). *E. coli* AW1.7 had higher heat resistance in ground beef with and without 5.6% seasoning binder compared to *S. enterica* ATCC 43845. The addition of 5.6% seasoning binder increased the *D*-values of *E. coli* AW1.7 in ground beef. At 55 and 65°C a trend (p-value <0.10) indicated that the addition of 5.6% seasoning binder increased the *D*-values of *E. coli* AW1.7. Interestingly, there were no significant differences (p-value <0.05) in the D-values of *S. enterica* ATCC 43845 when heated in ground beef prepared with or without 5.6% seasoning binder, with the exception of 67.5°C.

Figure 3: (A) Typical survivor curve for *E. coli* AW1.7 in ground beef (24% fat) at 55°C. (B) Typical survivor curve for *S. enterica* ATCC 43845 in ground beef (24% fat) at 55°C.

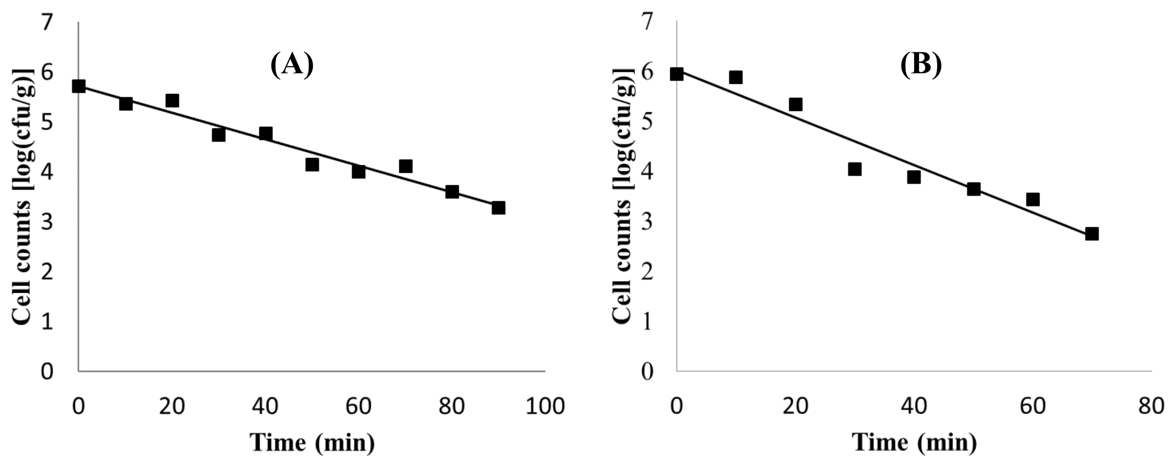

Table 6: *D*-values for *E. coli* AW1.7, *S. enterica* ATCC 43845 in ground beef heated to temperatures between 55 and 70°C. Data are shown as means ± standard deviation of three replicates. Differences between means within a strain (p-value <0.05) without and with 5.6% seasoning binder are indicated by different superscripts.

Temperature (°C)	*D*-value (min)			
	***E. coli* AW1.7**	***E. coli* AW1.7 with 5.6% seasoning binder**	***S. enterica* ATCC 43845**	***S. enterica* ATCC 43845 with 5.6% seasoning binder**
55	33.7 ± 6.5	39.8 ± 2.6	21.1 ± 3.2	26.5 ± 2.7
57.5	16.9 ± 4.8[a]	29.3 ± 7.6[b]	9.1 ± 1.0	11.4 ± 1.4
60	4.7 ± 0.7[a]	7.3 ± 1.9[b]	4.2 ± 0.2	4.0 ± 0.4
62.5	2.7 ± 0.4[a]	4.0 ± 0.8[b]	2.2 ± 0.5	2.7 ± 0.2
65	0.7 ± 0.2	1.0 ± 0.3	0.5 ± 0.1	0.6 ± 0.1
67.5	0.2 ± 0.03[a]	0.5 ± 0.1[b]	0.2 ± <0.01[a]	0.3 ± 0.01[b]
70	0.1 ± 0.01[a]	0.2 ± 0.02[b]	0.1 ± 0.05	0.2 ± 0.03

3.2 Effect of seasoning binder z-values of *E. coli* AW1.7 and *S. enterica* ATCC 43845 in ground beef

To determine the effect of seasoning binder on *z*-values of *E. coli* AW1.7 and *S. enterica* ATCC 43845 in ground beef, the z-values were calculated based on the previously determined *D*-values (Table 3). The *z*-values for *E. coli* AW1.7, without and with 5.6% seasoning binder, and *S. enterica* ATCC 4384, without and with 5.6% seasoning binder, were 5.8 and 6.0, 6.4 and 6.6, respectively (Figure 4). The thermal resistance curves had correlation coefficients greater than 0.98 (Figure 4).

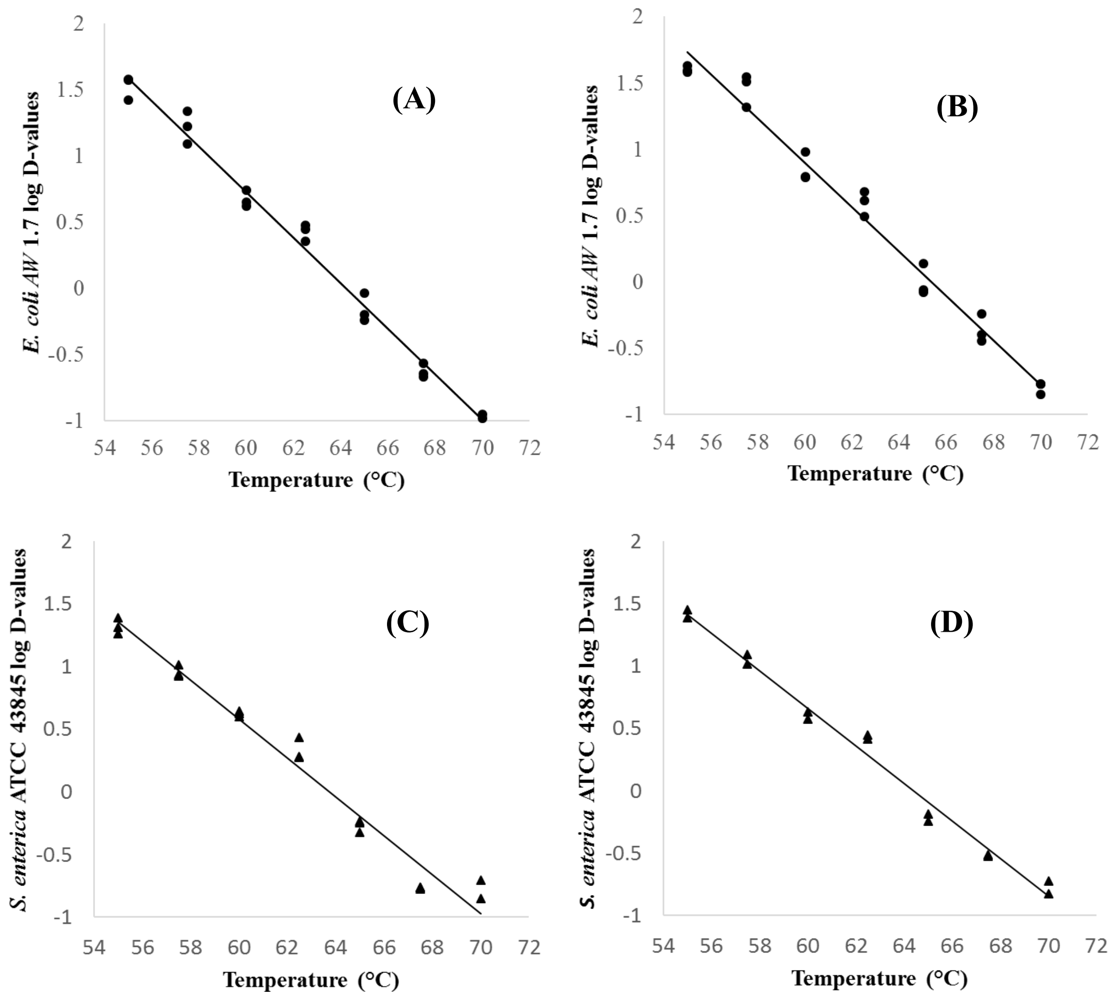

Figure 4: Thermal reduction curves (log D-value versus temperature [°C]) used to determine z-values for (A) *E. coli* AW1.7: z-value of 5.8 in ground beef, with a correlation coefficient of 0.994. (B) *E. coli* AW1.7: z-value of 6.0 in ground beef with 5.6% seasoning binder, with a correlation coefficient of 0.994. (C) *S. enterica* ATCC 43845: z-value of 6.4 in ground beef, with a correlation coefficient of 0.988. (D) *E. coli* AW1.7: z-value of 6.6 in ground beef with 5.6% seasoning binder, with a correlation coefficient of 0.994.

3.3 Survival of heat-resistant and heat-sensitive organisms in cooked ground beef patties

3.3.1 Survival of *E. coli* and *S. enterica* in ground beef patties stored for 48 h and cooked to a core temperatures of 71°C

To assess the effect of the addition of seasoning binder on survival heat-resistant and heat-sensitive organisms after 48 h storage at refrigeration temperature, LHR-positive and -negative *E. coli* and *S. enterica* strains were inoculated into beef patties without and with 5.6%

seasoning binder and stored at 4°C for 48 h. *E. coli* AW1.7 pHR1 and *S. enterica* ATCC 13311 were used as a heat-sensitive reference strains. There was no significant difference between the reductions of either LHR-positive and -negative *E. coli* and *S. enterica* strains without and with 5.6% seasoning binder (Figure 5). The addition of seasoning binder and storage at 4°C for 48 h did not have a significant effect on survival during cooking. However, it is worth nothing the addition of seasoning binder did have a significant effect on the cooking time of ground beef patties and increased the mean cook time by 30 sec (data not shown). It was observed that the probe in the geometric center of the beef patty was not always the last probe to reach the desired internal temperature.

3.3.2 Survival of *E. coli* AW1.7 in ground beef patties cooked to core temperatures of 63°C, 71°C, and 74°C

To assess the effect of different end-point core temperatures on the survival of *E. coli* AW1.7, ground beef patties inoculated with *E. coli* AW1.7 were cooked to an internal temperature of 63, 71, or 74°C. When cooked to 63 and 71°C cell counts of the heat-resistant *E. coli* AW1.7 were reduced by 1.6-log (CFU/g) and 3.3-log (CFU/g), respectively (Figure 6). When cooked to 74°C cell counts of the heat-resistant *E. coli* AW1.7 were reduced by greater than 5-log (CFU/g). There were significant differences in cell reduction between burgers cooked to an internal temperature of 63, 71, or 74°C.

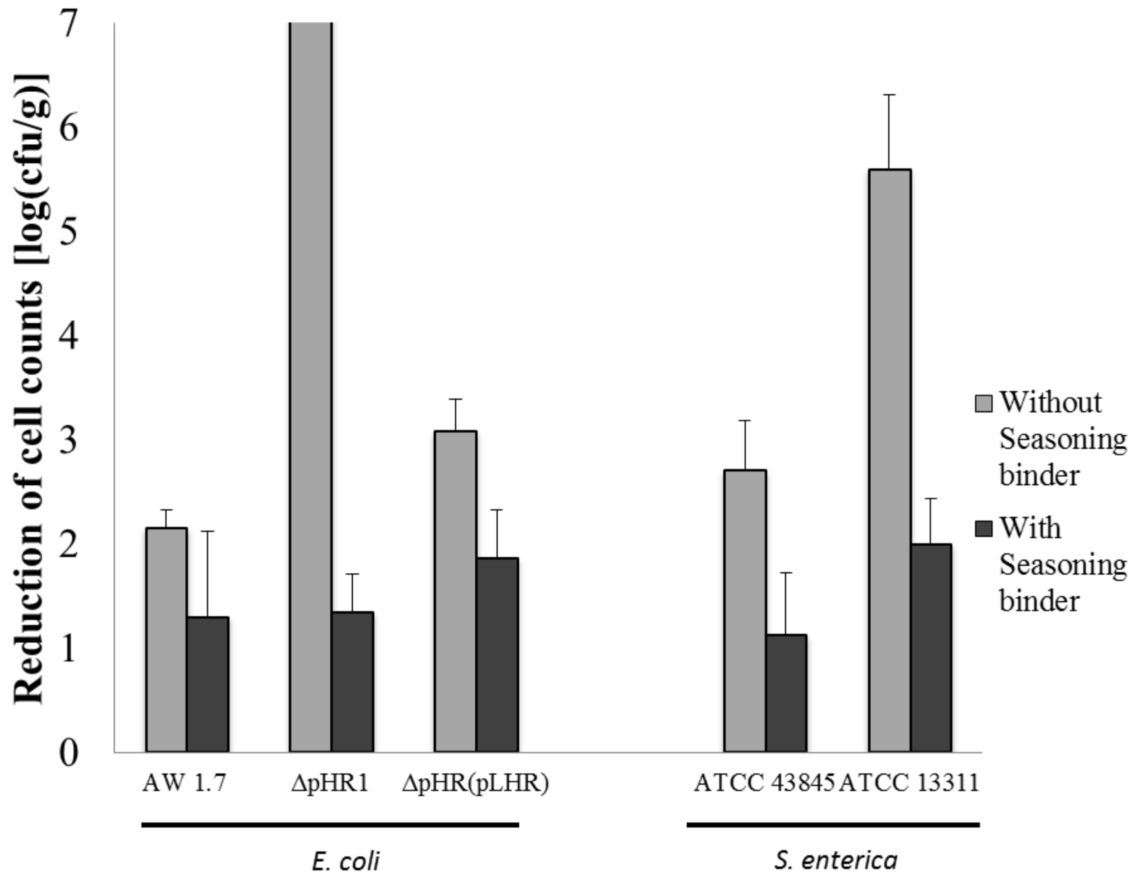

Figure 5: Thermal inactivation of *E. coli* and *S. enterica* in ground beef patties, without and with 5.6% seasoning binder. Patties were stored at 4°C for 48 h prior to cooking to an internal temperature of 71°C. Reduction of cell counts for LHR-positive and LHR-negative *E. coli* and *S. enterica* was determined with 3 biological replicates and means ± standard deviations are shown.

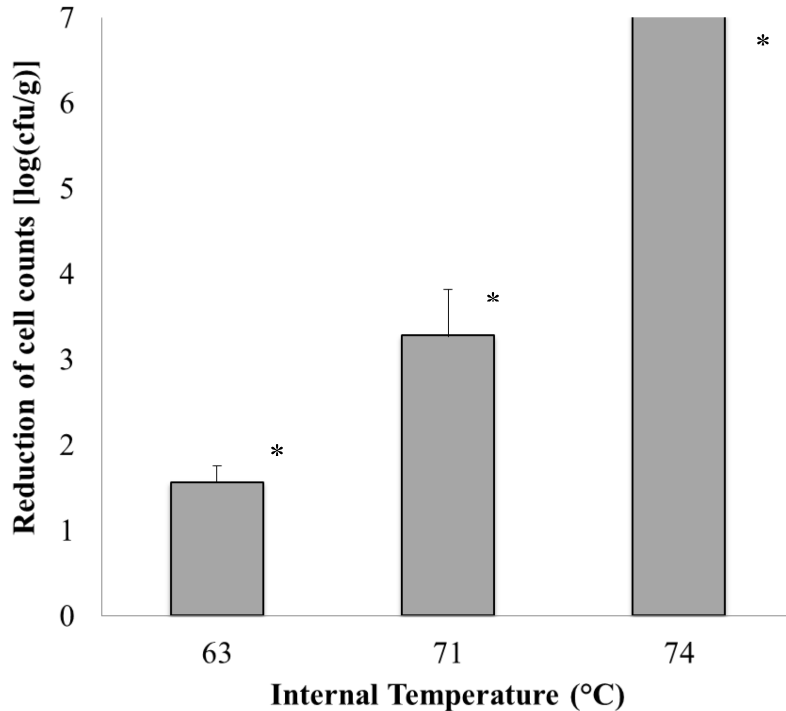

Figure 6: Thermal inactivation of *E. coli* AW1.7 in ground beef patties cooked to an internal temperature of 63, 71, or 74°C. Reduction of cell counts was determined with 3 biological replicates and means ± standard deviations are shown. Statistically significant (p-value <0.05) differences of survival at different end point temperatures are indicated by an asterisk (*).

3.3.3 Survival of VTEC in ground beef patties cooked to a core temperature of 71°C

To assess the effect of seasoning binder (containing NaCl) on survival of VTEC during cooking of ground beef a 5-strain VTEC cocktail was inoculated into beef patties with 0, 5.6 and 16.8% seasoning binder added to the patties, which represents *ca* 0, 1 and 3% NaCl. Patties were cooked to an internal temperature of 71°C, in accordance with Health Canada's recommended cooking temperature. At 5.6% and 16.8% added seasoning binder, the cocktail of VTEC survived better as compared to survival in patties without added seasoning binder (Figure 7). Cell counts of VTEC decreased greater than 5-log (CFU/g) in the absence of seasoning binder and about 2.8 log (CFU/g) in the presence of 5.6% seasoning binder. The increased survival with

the addition of 16.8% seasoning binder was comparable to the effect of the addition of 5.6% seasoning binder.

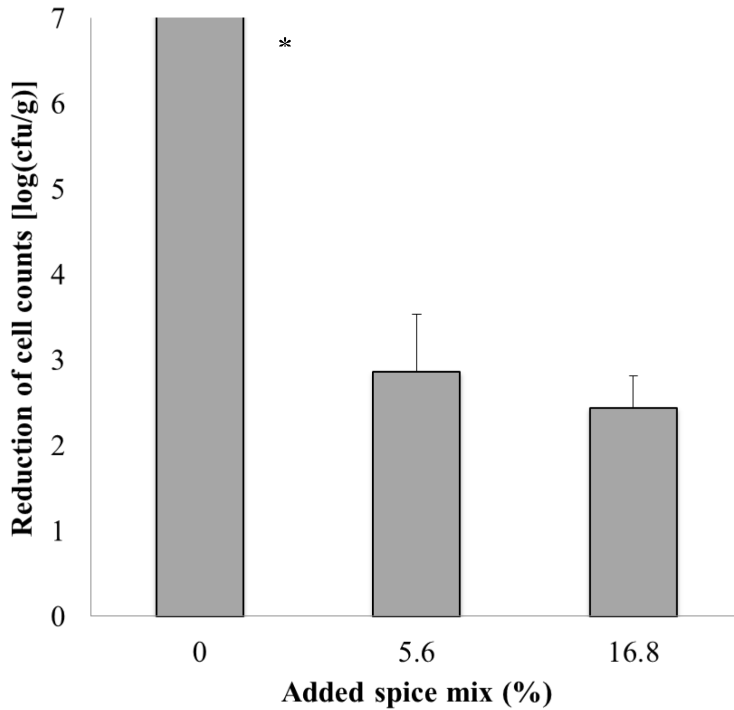

Figure 7: Thermal inactivation of VTEC cocktail in ground beef patties with varying levels of seasoning binder cooked to an internal temperature of 71°C. Reduction of cell counts for VTEC cocktail was determined with 2 biological replicates and means ± standard deviations are shown. Statistically significant (p-value <0.05) differences of survival at among seasoning binder concentrations are indicated by an asterisk (*).

3.4 Effect of multiple thermocouples to measure end point temperature

To assess the effect of multiple thermocouples to measure end-point temperature (internal core temperature) multiple probes were used as shown in Figure 2. The temperature profile of all 4 thermocouples is shown in Figure 8. The probe in the geometric center of the ground beef patty

was not always the last probe to reach the desired end-point temperature.

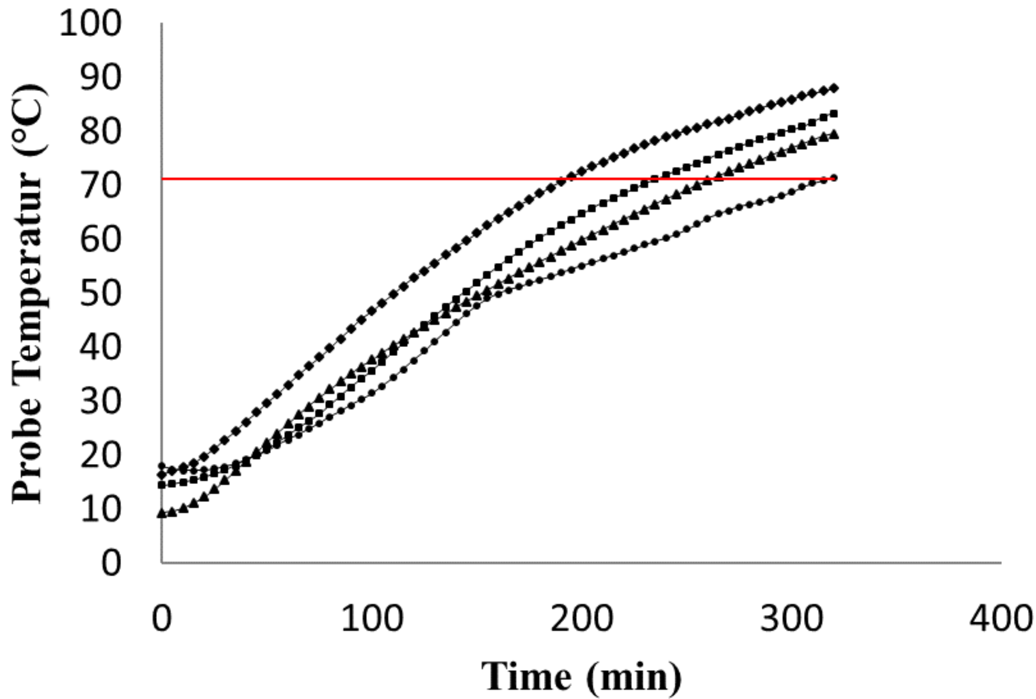

Figure 8: Temperature profile of a ground beef patty cooked to an internal temperature of 71°C. Profiles displayed are Probe 1 (▲), Probe 2 (■), Probe 3 (●), Probe 4 (♦). Horizontal line designates desired end point temperature of 71°C.

3.5 F-values of *E. coli* AW1.7 in ground beef

To assess the process lethality of *E. coli* AW1.7 in ground beef, the hypothetical and observed lethality were compared. This comparison, shown in Table 7, was done for ground beef patties inoculated with *E. coli* AW1.7 and cooked to an internal temperature of 63, 71, or 74°C. Cooking times ranged from 235 to 250 sec, 265 to 320 sec, or 305 to 355 sec for ground beef patties cooked to 63, 71, or 74°C, respectively. The F-value, expression of cooking time and internal temperature, is shown in Table 7. As the final internal temperature increased, the time to cook, theoretical log reduction, and experimental log reduction increased. The experimental log

reduction was lower for burgers cooked to an internal temperature of 63 and 71°C. However, the theoretical log reduction was higher for burgers cooked to an internal temperature of 74°C.

Table 7: Process lethality determination of *E. coli* AW1.7 in ground beef patties (24% fat) cooked to different core temperatures. Process lethality calculated with reference temperature (70°C), *z*-value (5.8°C), and D_{70} (0.1045 min). Data are shown as means ± standard deviation of three replicates.

Internal Temperature (°C)	Time to Cook (sec)	F-value (min)	Theoretical Log Reduction	Experimental Log Reduction
63	243	0.02	0.17	1.56
71	292	0.38	3.67	3.27
74	326	1.61	15.39	> 7

3.6 Survival of heat-resistant *E. coli* and *S. enterica* in chicken nuggets cooked to a core temperature of 74°C

To assess the survival of heat-resistant organisms in heated non-RTE products, heat-resistant strains were inoculated into chicken nuggets, partially deep-fried and frozen. The nuggets were cooked to an internal temperature of 74°C as per Health Canada recommendations (Health Canada, 2014). *E. coli* AW1.7 pHR1 was used as a heat-sensitive reference strain. Cell counts of the heat-resistant *E. coli* AW1.7, *E. coli* FUA 1675, and *S. enterica* ATCC 43845 were reduced by 2.3, 2.1, and 2.4, respectively (Figure 9). In contrast, counts of the heat-sensitive *E. coli* AW1.7 pHR1 were reduced by more than 5-log (CFU/g).

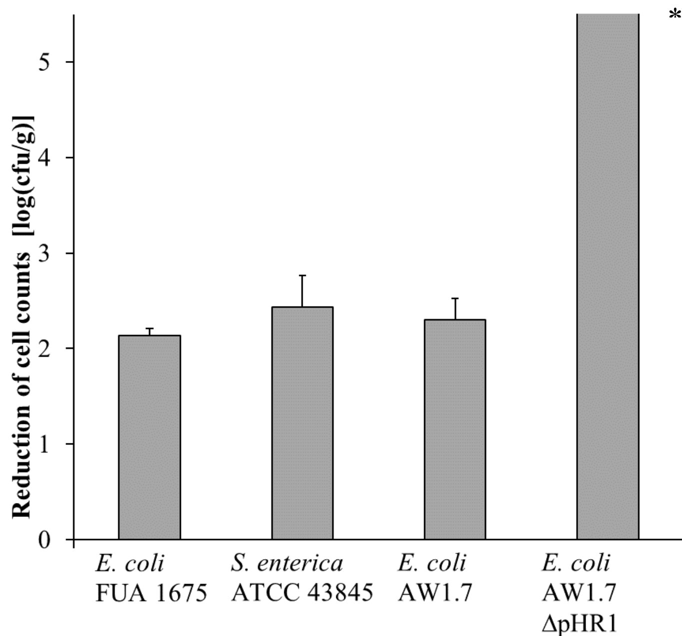

Figure 9: Thermal inactivation of *E. coli* and *S. enterica* in chicken nuggets cooked to an internal temperature of 74°C. Reduction of cell counts was determined with 3 biological replicates and means ± standard deviations are shown. Statistically significant (p-value <0.05) differences among organisms are indicated by an asterisk (*).

3.7 F-values of *E. coli* AW1.7 and *S. enterica* ATCC 43845 in chicken nuggets

To assess the process lethality of *E. coli* AW1.7 and *S. enterica* ATCC 43845 in chicken nuggets, the hypothetical and observed lethality were compared. This comparison, shown in Table 8, was done for heated and frozen chicken nuggets inoculated with *E. coli* AW1.7 and *S. enterica* ATCC 43845, and cooked to an internal temperature of 74°C. The ground beef reference values were used to assess if ground beef values could predict process lethality in chicken nuggets from ground beef data. The theoretical log reduction in chicken nuggets were substantially higher than the experimental.

Table 8: Estimated process lethality determination of *E. coli* AW1.7 and *S. enterica* ATCC 43845 in chicken nuggets cooked to an internal temperature of 74°C. Process lethality calculated with reference values from ground beef (24% fat) with seasoning binder. For *E. coli* AW1.7 a reference temperature of 70°C, *z*-value of 6.0°C, and D_{70} of 0.17 min was used. For *S. enterica* ATCC 43845 a reference temperature of 70°C, *z*-value of 6.4°C, and D_{70} 0.16 min was used. Data are shown as means ± standard deviation of three replicates.

Organism	Time to Cook (sec)	F-value (min)	Theoretical Log Reduction	Experimental Log Reduction
E. coli AW1.7	232	1.23	7.0	2.3
S. enterica ATCC 43845	228	0.99	5.8	2.4

3.8 Water activity of different meat matrices

Table 9 shows the difference in water activity between ground chicken breast and chicken nugget batter and between ground beef with and without seasoning binder. The addition of seasoning binder to both protein matrices reduced the water activity. The effect is greater in ground beef than in ground chicken. Ground beef has a higher percentage of seasoning binder and has a lower water activity.

Table 9: Water activity of meat matrices prior to cooking

Meat Matrix	a_w
Ground chicken breast	0.988
Chicken nugget batter (2.5% seasoning binder)	0.981
Ground beef (24% fat)	0.988
Ground beef (24% fat; 5.6% seasoning binder)	0.969

4. Discussion

E. coli AW1.7 and *S. enterica* ATCC 43845 are exceptionally heat-resistant bacteria that contain the LHR and differ from heat sensitive, LHR-negative strains (Mercer et al., 2015). The aim of this study was to determine the effects of seasoning binder on heat resistance and degree of thermal inactivation of *E. coli* and *S. enterica* in food matrices.

4.1 Influence of the seasoning binder on thermal inactivation of heat resistant bacteria

As thermal processing is often utilized in the food industry to control pathogens in food, it is important to use exceptionally heat-resistant strains to identify the range of time-temperature guidelines needed to ensure product safety. Additionally, evaluating the effect of components in food is critical as they play a role in heat resistance and influence survival.

While the thermal inactivation results of this study followed log linear inactivation kinetics, several other models have been used to determine inactivation. Several non-linear models have been published when thermal treatment was used in combination with a secondary treatment (Juneja et al., 2013). However this was not considered for this thesis work as a secondary treatment was not used and the survival curves fit log linear model.

CFIA requires a 5-log reduction of *E. coli* O157:H7 in ground beef products and 6.5-log reduction of *Salmonella* in meat products not containing poultry. The values in Table 10 can be used to predict the time required at specific temperatures to achieve a 5-log reduction of heat-resistant *E. coli* AW1.7 in regular ground beef with and without seasoning binder. The values in Table 11 can be used to predict the time required at specific temperatures to achieve a 6.5-log reduction of the heat-resistant *S. enterica* ATCC 43845 in regular ground beef with and without seasoning binder. Regular ground beef should be heated to an internal temperature of 70°C for at

least 32 sec and 58 sec to achieve adequate reductions of *E. coli* AW1.7 and *S. enterica* ATCC 43845, respectively. These recommendations are based on the *D*-values calculated and reported in this thesis. It is important to note these times are minimum processing times after the target temperature has been reached. These recommendations are based on CFIA's guidelines dictating a 5-log reduction of *E. coli* O157:H7 in beef and 6.5-log reduction of *Salmonella* in meat products not containing poultry. Thermal death time values from this thesis will assist food processors in determining acceptance limits for critical control points to ensure adequate destruction of heat resistant *E. coli* and *Salmonella* in cooked beef. These values offer a reference that establishments can use to provide scientific justification to CFIA for their processes but they should also provide additional data to support their processes.

Currently CFIA's Annex D Table 1 cooking time/temperature guidelines for non-poultry containing meat products does not consider any differences in food matrices composition. CFIA's Annex D Table 2 and 3 cooking time/temperature guidelines for products containing chicken and turkey, respectively, do account for differences in fat percentage but not salt content.

Table 10: Minimum time at different temperatures (minimum dwell time) needed to obtain a 5D lethality of heat resistant *E. coli* AW1.7 in regular ground beef

	***E. coli* AW1.7**	
Temperature (°C)	**0% Seasoning binder**	**5.6% Seasoning binder**
55	169 min	200 min
57.5	85 min	147 min
60	24 min	37 min
62.5	14 min	20 min
65	4 min	6 min
67.5	72 sec	134 sec
70	32 sec	48 sec

Table 11: Minimum time at different temperatures (minimum dwell time) needed to obtain a 6.5D lethality of heat-resistant *S. enterica* ATCC 43845 in regular ground beef

	***S. enterica* ATCC 43845**	
Temperature (°C)	**0% Seasoning binder**	**5.6% Seasoning binder**
55	138 min	172 min
57.5	60 min	74 min
60	28 min	27 min
62.5	14 min	18 min
65	4 min	5 min
67.5	67 sec	119 sec
70	58 sec	67 sec

Earlier studies reported the heat resistance of *E. coli* AW1.7 in LB broth (D_{60} = 71 min; Dlusskaya et al., 2011) and *S. enterica* ATCC 43845 in buffer (D_{57} = 31 min; Ng et al., 1969). The reported D_{60} of *E. coli* AW1.7 is higher than presented in this thesis and could be attributed to differences between broth and meat matrices. The *D*-values of *E. coli* AW1.7 in 24% fat from this thesis are substantially higher than the published *D*-values of *E. coli* O157:H7 in 19.1% fat ground beef (Smith et al., 2001) and 10% fat ground beef (Juneja et al., 1997). The D_{60} determined in the current research of 4.2 for *S. enterica* ATCC 43845 is in agreement with the D_{61} reported by Smith et al. (2001) in ground beef at 19.1% fat. The *D*-values of *S. enterica* ATCC 43845 in 24% fat obtained in the current research are higher than those determined by Murphy et al. (2002) for a 6 serotype cocktail that included *S. enterica* ATCC 43845. This was to be expected as the other serotypes used in the cocktail were not as heat resistant as the strain of *S.* Senftenberg ATCC 43845.

As demonstrated by the current research, and reported by numerous other studies (Ahmed et al., 1995; Juneja et al., 1997; Smith et al., 2001; Murphy et al., 2002) the composition of meat

influences the thermal inactivation of pathogens. It is recommended that regulatory agencies assess their guidelines for processors to account for these factors.

4.2 Effect of seasoning binder and storage on thermal reduction

Rode (2016) reported higher survival of heat-sensitive and heat-resistant *E. coli* in ground beef patties containing 3% NaCl than in patties with no NaCl. In the current study, this effect was not observed for heat-sensitive or heat-resistant *E. coli* and *S. enterica* in ground beef patties with 5.6% seasoning binder when stored for 48 h and cooked. This could be due to the confounding variable of the seasoning binder, which contained binders that increased moisture retention during cooking, thus increased the time to reach target temperatures. The cells may have survived better but they were heat treated longer, resulting in increased lethality.

During growth at increased salt concentrations, a significant uptake in glucose and trehalose occurs (Pleitner et al., 2012). Accumulation of compatible solute is linked to increased heat resistance (Ruan et al., 2011). During refrigeration storage, you could expect acquisition of compatible solutes either through diffusion or synthesis.

Previous work by Mercer et al. (2017) used a single temperature probe in the geometric center of beef patties to confirm the final core temperature of beef patties during cooking on a clamshell grill. In the current study, multiple probes were used to determine end point temperatures. Slight variance among the survival of heat-resistant *E. coli* and *Salmonella* in ground beef patties cooked to 71°C observed by Mercer et al. (2017) and this thesis may be attributed to this closer monitoring of core temperature. In addition, it is valuable to have real time temperature readings and the ability to analyze recorded temperature over time. Temperature monitoring is essential for meaningful results in experiments when considering the

time-temperature relationship of thermal inactivation. To achieve this level of temperature monitoring, 4 probes connected to thermocouples were used. During cooking, differential heating of the ground beef patties may occur. In the current study, the probe in the geometric center of the beef patty was not always the last probe to reach the desired internal temperature.

Research conducted for this thesis used a retail clamshell grill to cook the patties which simulates methods used in commercial settings; however, the method of cooking may influence thermal inactivation. Murphy et al. (2001) observed that humidity plays a role in survival of *Salmonella*. They demonstrated that cooking patties inoculated with *Salmonella* in a pilot-scale air convection oven increased the survival by 2-log when cooked at low humidity. In the current experiment, humidity was not monitored during the cooking of the patties. It may be possible that the reduction of heat-resistant *Salmonella* could be achieved when cooked a high humidity environment. It is worth noting that in this thesis burgers were cooked individually. This is not entirely reflective of a real world scenario where multiple patties would be cooked simultaneously. Murphy et al. (2001) observed increased survival of *Salmonella* when patties were touching or overlapping during cooking. In that case the results from the current research where patties were cooked individually would under estimate survival compared to a commercial setting.

4.3 Effect of internal core temperature on thermal reduction of *E. coli* AW1.7

As expected, the higher the final internal core temperature, the higher the reduction of *E. coli* AW1.7. A temperature of 63°C was chosen to reflect an undercooked patty. The temperature of 71°C was chosen to mirror Health Canada's recommended internal temperature for non-intact meats. Finally, 74°C was chosen as a higher than recommended temperature to reach a 5-log reduction of a heat resistant *E. coli*.

The relationship between temperature and process lethality of *E. coli* AW1.7 in a patty cooked to 71°C compared over time is shown in Figure 10. When calculating the process lethality, the data from the thermocouple with the lowest temperature reading data was used to get the most conservative value. This is justified as the coldest spot in the food product has the slowest transfer of heat and therefore greatest area of concern for survival during thermal processing. The geometric center of the patty was not always the coolest spot in the patty, thus the 4 probes were used in the current research. This could be due to heat distribution from the heating elements, uneven contact of the patty with the surface of the top plate of the clam shell grill, or uneven thermal kinetics of the meat matrix. The same ground beef used to determine the *D*- and *z*-values was used to form the ground beef patties, thus the *D*- and *z*-values were used to calculate the theoretical process lethality. This was to ensure the consistent physical properties and fat content between the two experiments.

The process lethality is affected by the numerous processing parameters including the rate of heat transfer, and thermal and physical properties that affect rate of heat transfer in patty. For all the burger experiments in the current research, the grill temperature was kept constant. To ensure consistency, the same ground beef was used for patty formulations and formed in the same patty press to produce patties of uniform physical properties and maintain even heat transfer across all experiments. It is important to note that the process lethality is a calculated value and does not always reflect the experimental reduction. This is especially true in a food matrix that does not undergo isothermal heating.

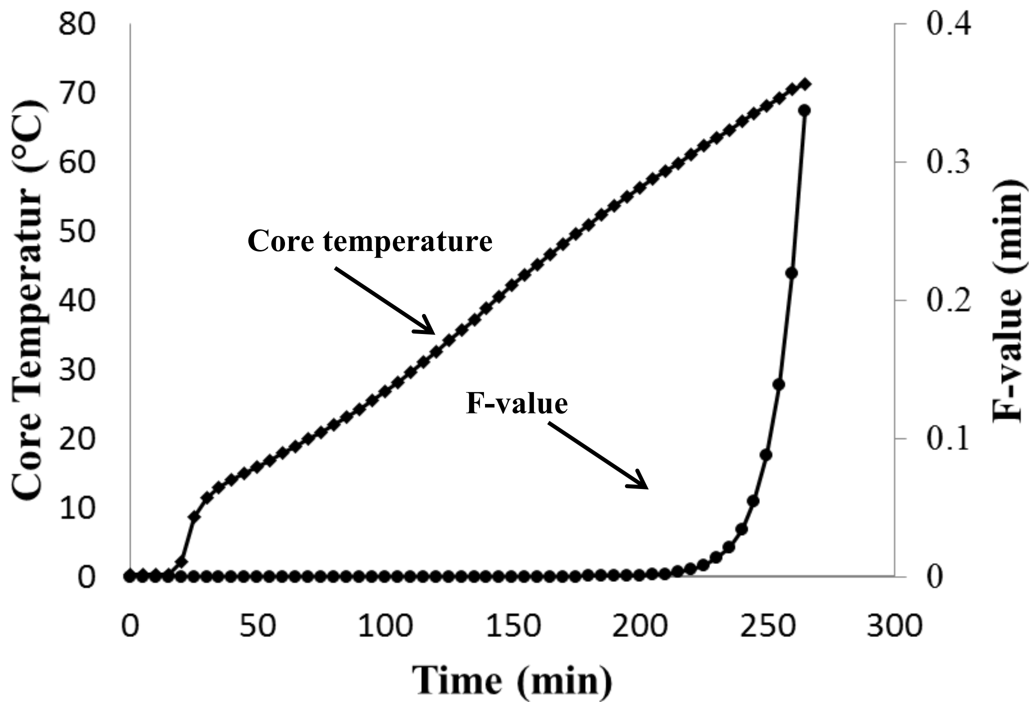

Figure 10: The core temperature and process lethality of *E. coli* AW1.7 in a ground beef patty when cooked to an internal temperature of 71°C. Core temperature (♦) and F-value (●). F-value calculated with a reference temperature of 70°C and *z*-value of 5.8°C.

4.4 Influence of the seasoning binder on reduction of a cocktail of VTEC

Pathogenic *E. coli* are not renowned for their heat resistance. Kaur et al. (1998) reported that reducing the water activity from 0.995 to below 0.980 resulted in increased heat resistance in *E. coli* O157:H7. In the current research, seasoning binder reduced the water activity of the ground beef matrix below 0.980. The results from the current research are in line with those of Kaur et al. (1998) who found that heat-sensitive pathogens have increased heat resistance and survival at slightly reduced water activity. This finding is concerning as increased salt or seasoning binder in ground beef now presents a more substantial risk as they will increase the survival of pathogenic *E. coli*.

4.5 Strain variation and survival in cooked breaded and frozen chicken nuggets

Heated NRTE meat products have recently been of concern as *Salmonella* has been isolated in finished product (PHAC, 2017). This research confirmed that *E. coli* and *Salmonella* were able to survive in fully cooked breaded and frozen chicken nuggets. The chicken nugget preparation and product in this study was comparable to commercial chicken nugget preparation and retail product available to consumers. The strain *E. coli* FUA 1675 used in this study, and that was able to survive cooking, was isolated from a finished product by a food processor. After a 30 sec par-fry the nuggets had the visual appearance of being cooked with a golden-brown breading. In heated NRTE meat products, such as chicken nuggets and chicken strips, there is a risk that pathogens will survive the initial rapid cooking that is done to "set" the batter. This risk is increased as many consumers do not consider these products as raw and some use inappropriate cooking methods prior to consumption of the product (MacDougall et al., 2004). Bucher et al. (2008) examined survival of *Salmonella* isolated from raw chicken nuggets and concluded that adequate cooking to 71°C eliminated the hazard. However, based on results of the current research, the survival of heat resistant strains in heated and frozen chicken nuggets cooked to 74°C presents an additional risk to consumers even when the product is fully cooked.

Juneja et al (2001) reported higher *D*-values of a 6-serotype *Salmonella* cocktail at 55°C to 70°C for chicken tenders (21.43% fat) compared to beef patties (18.56% fat). This is in agreement with the estimation of process lethality in chicken nuggets using *D*-values of beef presented in this thesis. The process lethality in chicken nuggets could not be predicted using the *D*-values from the results with ground beef. The predicted lethality was far greater than observed lethality and thus was not suitable for comparison.

The onus for the solution and reducing the risk for consumers is in part on the processor and the consumer. Unfortunately, Canadian processors are at a disadvantage as CFIA does not accept a dose cumulative approach, in that the thermal lethality must be achieved in a single dose. It has been recommended by several other authors that a careful review of the manufacturing process should be undertaken identify interventions that could reduce the risk of survival presented by these products (Hobbs et at., 2017; MacDougall et al., 2017, Catford et al., 2017). This could mean processors have to change their formulation in a manner that is more receptive to lethality or reducing survival. Consumers need to be aware that heat treated non RTE meat products do need to be cooked and not simply reheated. Like ground beef patties consumers need to utilize thermometers to ensure that their food is reaching the correct internal temperature.

4.6 Study Limitations

Data on the difference between ground beef patties with either seasoning binder or salt would have been useful. That would have elucidated if the NaCl in the seasoning binder was solely responsible for differences observed in heat resistance or if the combination effect of salt and lowered water activity due to the presence of binders had a greater effect on survival.

It would have been interesting to determine the *D*- and *z*-values of *E. coli* AW1.7 and *S. enterica* ATCC 43845 in chicken nuggets and make comparisons between ground chicken and chicken nugget batter, and between fresh and frozen chicken nugget batter. This would be useful in more accurate in predicting process lethality. More research on the heat treatment in ground chicken it would have been useful to determine what internal temperature is required for a 7-log reduction of *Salmonella* in chicken nuggets. Additionally, it would be interesting to examine the reduction of a cocktail of heat-resistant and heat-sensitive *Salmonella* as a cocktail would be

more indicative of real world processing. Additionally, it would have been interesting to note differences of thermal inactivation values and thermal reduction in ground beef with a fat content lower than 24%.

4.7 Conclusion

This study demonstrated that seasoning binder has some influence on increasing the thermal inactivation kinetics of *E. coli* AW1.7 in ground beef.

It is important to understand both the genetics of heat resistance in *E. coli* and *Salmonella* and how that resistance in meat products influences survival. The current regulatory recommendation of cooking to 71°C does not result in the required 5-log reduction for heat-resistant *E. coli*. Similarly, the current recommendation of cooking non RTE poultry products to 74°C does not result in the desired 7-log reduction in heat resistant *Salmonella*. These results provide the scientific support for a change to the current regulatory standards and recommendations on cooking of meat products. Together these findings are meant to provide guidance to improve current processing practices to prevent survival of pathogenic *E. coli* and *Salmonella* in heated meat products, and ensure the safety of the Canadian food supply.

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