Can Foodservice Packaging be Used to Prevent Foodborne Illnesses: A Comparative Study of Multiple Packaging

A Thesis Presented to the
Faculty of the
Conrad N. Hilton College of Hotel and Restaurant Management
University of Houston

In Partial Fulfillment
Of the Requirements for the Degree
Master of Science

Megan Marie Protz
May 2016

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Dedication

This thesis is dedicated to the most important people in my life: my parents, best friend, and loving dog. Thank you to my parents for supporting me through years of schooling, a cross state move, and molding me into the individual I am today. Thank you to my best friend for guiding me through this journey, and maintaining my sanity along the way. Lastly, thank you to my adorable dog, for being the best emotional support despite the length of hours spent away from home.

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Abstract

A comparative study of multiple packaging was conducted to determine if foodservice packaging could be used as an intervention in preventing microbial growth on leftovers that are temperature abused. Aerobic plate counts were used to establish a baseline of bacterial growth in comparison to cardboard, plastic, and Styrofoam foodservice packaging and various scenarios including fridge, counter, and incubator. Samples of cooked chicken were counted at six-hour intervals for twelve hours. Survival and growth of *Salmonella* Typhimurium 53647 in cardboard, plastic, and Styrofoam packaging was then analyzed over a twelve-hour timespan in the different environmental scenarios. Chicken portions stored at 2 to 37°C for 12 hours were inoculated with 2.58 log CFU/g of *Salmonella*, and counts were made at 6-hour intervals to determine the effect of packaging. Results concluded that there was a significant difference in bacteria growth overtime, and plastic foodservice packaging has the greatest significance for survival and growth of *Salmonella*. These findings suggest that select foodservice packaging may be used as a viable tool for reducing microbial populations and can help manage risk of human illness from food.

CHAPTER I

Introduction

The use of the ubiquitous 'to-go' box or 'doggy bag' containing leftovers from a meal is a frequent occurrence for most Americans who dine out. Sometimes these leftovers are left unattended while life carries on - they are left in the car or on countertops and tables before making their way into the trusted cold zone of the refrigerator. It begs the question: Why is it so important to cool foods, and how should they be adequately cooled so that they are safe to eat? Food safety is about managing risk and focusing on practices that address risk factors known to contribute to foodborne illness.

Overview

Throughout our lifetimes we are subjected to risks and hazards of all kinds. The food supply in the U.S. is one of the most abundant, nutritious, and safest on earth. However, there is no absolute degree of safety, not even for the food we consume (Banwart, 1989). The U.S. Food and Drug Administration (FDA) estimates that there are approximately 48 million cases of foodborne illness annually—the equivalent of sickening 1 in 6 Americans each year. Each year these illnesses result in an estimated 128,000 hospitalizations and 3,000 deaths (FDA, 2015).

With their busy lifestyles, many Americans purchase foods for convenience such as prepared salads and deli items that require more handling and thus have an increased opportunity for contamination. With the increasing complexity of food production processes, international distribution patterns, and changing consumption practices, foodborne pathogens such as *Escherichia coli* O157:H7 have emerged as important

causes of foodborne illness, and pathogens such as *Salmonella* have found new niches (Kasowski, Gackstetter, & Sharp, 2002).

According to the Department of Preventive Medicine and Biometrics, most foodborne illness is unrecognized, and reporting of suspected or identified foodborne illness is inconsistent at best (Kasowski et al, 2002). Although laboratory diagnosis of suspected foodborne illness occurs infrequently, there are enough data to suggest that it is a serious problem.

Most people do not like to waste or dispose of food that has not been eaten at the end of the meal. However, improper handling and storage of leftovers is one of the most common causes of food poisoning in the home. However, with care, it is possible to avoid both waste and illness (EUFIC, 2000). One fact that is important to remember is that once food has been cooked, it should be refrigerated or frozen within two hours. This includes the time the food is out of the refrigerator or oven before being served and the time it is on the table. If food is left at room temperature for more than two hours (one hour in hot temperatures), bacteria can grow to harmful levels, making it unsafe to eat (EUFIC, 2000).

Most foodborne illness outbreaks are a result of improper handling or contamination when meals are prepared. Sanitary food handling and proper cooking and refrigeration should prevent foodborne illnesses. USDA's Food Safety and Inspection Service (FSIS) has a zero tolerance for certain pathogens, including *Salmonella* and *Listeria monocytogenes*, in cooked and ready-to-eat products, such as chicken franks or lunch meat, that can be eaten without further cooking (USDA, 2016).

Salmonella enteritidis may be found in the intestinal tracts of livestock, poultry, dogs, cats, and other warm-blooded animals. This strain is only 1 of about 2,000 kinds of *Salmonella* bacteria; it is often associated with poultry and shell eggs. FSIS requires poultry establishments to meet *Salmonella* performance standards as a means of verifying that production systems are effective in controlling contamination by this pathogenic organism through agency inspection (USDA, 2016).

Problem Statement and Objectives

Infectious diseases spread through food or beverages are a common, distressing, and sometimes life-threatening problem for millions of people in the U.S. and around the world (U.S Department of Health and Human Services, 2015). The purpose of this study was to determine if foodservice packaging could be used as an intervention in preventing microbial growth on leftovers that are temperature abused. This is achieved by modeling growth behavior of microorganisms overtime and investigating whether foodservice to-go packaging can reduce this growth. The objective was to compare the performance of plastic, cardboard, and Styrofoam foodservice packaging in the prevention of bacterial growth. Significant results may justify food establishments using the foodservice packaging with least bacterial growth. It is believed that this causal research could lead to a development of more specialized techniques for antimicrobial films in food packaging.

Hypotheses

Hypothesis 1.

The use of foodservice packaging decreases bacteria counts in prepared foods.

Hypothesis 2.

The use of non-porous packaging materials decreases bacteria counts in stored food items.

Hypothesis 3.

The survival and growth of *Salmonella* onto chicken samples will vary between styles of packaging.

Justifications

Restaurants may be unfairly blamed for foodborne illnesses. The mismanagement of time and temperature guidelines for food results in excess microbial growth that ultimately leads to foodborne illnesses. This study seeks to shift the burden of responsibility from the foodservice provider to individual customers. Alternatively, when comparatively testing multiple packaging, results may indicate that bacterial growth favors one style of foodservice packaging over the other. If this yields true, restaurants should use the style of foodservice packaging with the least bacterial growth to protect the consumer from foodborne illness.

CHAPTER II

Literature Review

A thorough literature review was conducted to ensure reliability and validity during research. Several types of articles were reviewed and examined to create a framework for innovative study. Topics include food safety practices, foodborne illnesses, and foodservice packaging.

Food Safety Practices

Ensuring safe food is an important public health priority. For years, regulatory and industry food safety programs have focused on reducing the incidence of foodborne illness. The common goal of operators and regulators of food service establishments is to produce safe, quality food for consumers. Many people share the responsibility of providing safe food to the consumer in every stage of the production of food, including consumers, themselves. Since most consumers receive their food from retail and food service establishments, a significant share of the responsibility for providing safe food to the consumer rests with these facilities (FDA, 2009). Operators of food service establishments can make the greatest impact on food safety.

When cooling, cold holding, and date marking are viewed in the context of a total food safety system, the potential for bacteria growth increases with each uncontrolled process step (Brown et al, 2012). It is essential that each process step be routinely monitored in a manner that enables the manager to take prompt corrective actions before an unsafe product reaches the consumer. Full service restaurants had the greatest percentage of observations out of compliance for risk factors in a 2009 FDA report (See

Table 1). Factors included: time and temperature abuse, poor personal hygiene, contaminated equipment, inadequate cooking, and food from an unsafe source.

Table 1

Occurrence of Foodborne Illness Risk Factors in Full Service Restaurants

Foodborne Illness Risk Factor	# Out	Total Obs. (In & Out)	% Out
Improper Holding/Time & Temperature	261	477	54.7
Poor Personal Hygiene	195	477	40.9
Contaminated Equipment	165	471	35.0
Other/Chemical	26	103	25.2
Inadequate Cooking	35	227	15.4
Food From Unsafe Sources	29	242	12.0

Note. Adapted from *FDA Report on Occurrence of Foodborne Illness Risk Factors in Selected Institutional Foodservice, Restaurant, and Retail Food Store Facility Types*, 2009.

Food safety hazards are biological, chemical, or physical agents that cause illness or injury in the absence of their control, resulting in a food to be unsafe for human consumption (FDA, 2009). Biological hazards include bacterial, viral, and parasitic microorganisms. Chemical hazards may be naturally occurring or may be added during the processing of food. High levels of toxic chemicals may cause acute cases of foodborne illness, while chronic illness may result from low levels. Physical hazards can result from contamination or poor procedures at many points in the food chain from harvest to consumer, including those within the food establishment.

HACCP (Hazard Analysis and Critical Control Point) is a system that helps food business operators look at how they handle food and introduces procedures to make sure

the food produced is safe to eat (FDA, 2009). A critical control point (CCP) is a point or procedure in a specific food system where loss of control may result in an unacceptable health risk (FAO, 2015). Control can be applied at this point and is essential to prevent or eliminate a food safety hazard or reduce it to an acceptable level. Each CCP will have one or more control measures to assure that the identified hazards are prevented, eliminated, or reduced to cromulent levels. See Figure 1.

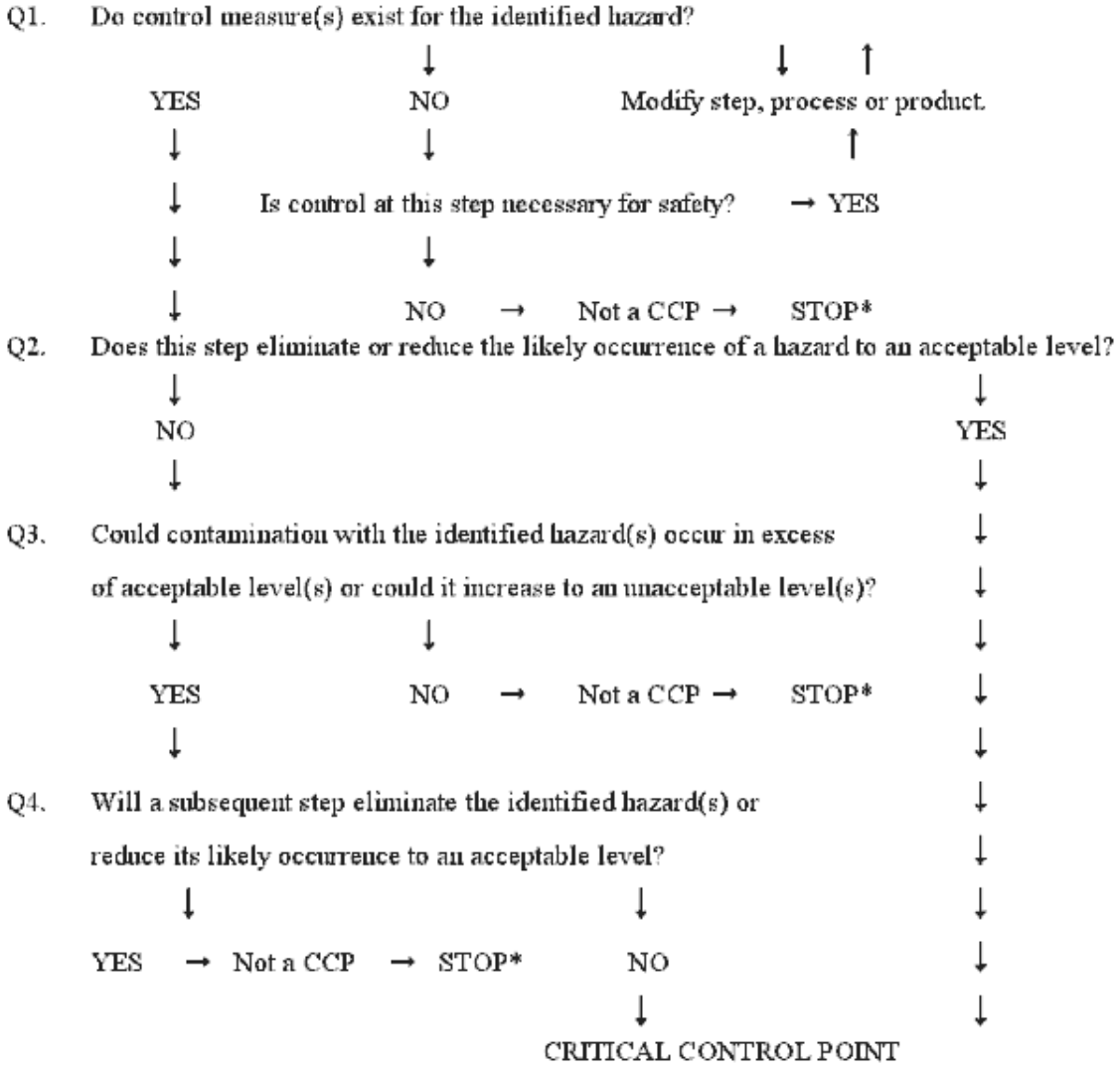

Figure 1. CCP Flowchart adapted from "Quality Assurance for Small-Scale Rural Food Industries," by P. Fellows, B. Axtell and M. Dillon, 1995, *Food and Agriculture Organization of the United Nations*.

Factors impacting food safety practices. Unsafe food preparation practices, such as improper glove use and not checking the temperatures of cooked, reheated, and cooled foods are common (Green & Selman, 2005). Epidemiological research has indicated that the majority of reported foodborne illness outbreaks originate in foodservice establishments, and case control studies have shown that eating meals outside the home is a risk factor for obtaining a foodborne illness (Green & Selman, 2005). Most outbreaks can be attributed to food workers improper food preparation practices (FDA, 2009). Findings indicate that improvement of restaurant workers food preparation practice is needed to reduce the incidence of foodborne illness.

Restaurant food cooling practices. Improper food cooling practices are a significant cause of foodborne illness. Most restaurant kitchen managers report that they have formal cooling processes and provide training to food workers on proper cooling (Centers for Disease Control and Prevention [CDC], 2012). However, many managers said they do not have tested and verified cooling processes, do not monitor time or temperature during cooling processes, or do not calibrate thermometers used for monitoring temperatures (CDC, 2012). Testing and verification should occur during initial development of the cooling process, involving measuring time and temperature on a routine basis.

Foodborne Illnesses

The epidemiology of foodborne illness is evolving. Major changes in food production, distribution, and consumption have created opportunities for new pathogens to emerge and for old ones to reemerge (Kasowski, Gackstetter, & Sharp, 2002).

The table below includes foodborne disease-causing organisms that frequently cause illness in the U.S. and illustrates that the threats are numerous and varied, with symptoms ranging from relatively mild discomfort to very serious, life-threatening illness. While the very young, the elderly, and persons with weakened immune systems are at greatest risk of serious consequences from most foodborne illnesses, some of the organisms shown below pose grave threats to all persons (FDA, 2015).

Table 2

Foodborne Disease Causing Organisms

ORGANISM	COMMON NAME OF ILLNESS	ONSET TIME AFTER INGESTING	SIGNS & SYMPTOMS	DURATION	FOOD SOURCES
Bacillus cereus	*B. cereus* food poisoning	10-16 hrs	Abdominal cramps, watery diarrhea, nausea	24-48 hours	Meats, stews, gravies, vanilla sauce
Campylobacter jejuni	Campylobacteriosis	2-5 days	Diarrhea, cramps, fever, and vomiting; diarrhea may be bloody	2-10 days	Raw and undercooked poultry, unpasteurized milk, contaminated water
Clostridium botulinum	Botulism	12-72 hours	Vomiting, diarrhea, blurred vision, double vision, difficulty in swallowing, muscle weakness. Can result in respiratory failure and death	Variable	Improperly canned foods, especially home-canned vegetables, fermented fish, baked potatoes in aluminum foil
Clostridium perfringens	Perfringens food poisoning	8–16 hours	Intense abdominal cramps, watery diarrhea	Usually 24 hours	Meats, poultry, gravy, dried or precooked foods, time and/or temperature-abused foods
Cryptosporidium	Intestinal cryptosporidiosis	2-10 days	Diarrhea (usually watery), stomach cramps, upset stomach, slight fever	May be remitting and relapsing over weeks to months	Uncooked food or food contaminated by an ill food handler after cooking, contaminated drinking water
Cyclospora cayetanensis	Cyclosporiasis	1-14 days, usually at least 1 week	Diarrhea (usually watery), loss of appetite, substantial loss of weight, stomach cramps, nausea, vomiting, fatigue	May be remitting and relapsing over weeks to months	Various types of fresh produce (imported berries, lettuce, basil)
***E. coli (Escherichia coli)* producing toxin**	*E. coli* infection (common cause of "travelers' diarrhea")	1-3 days	Watery diarrhea, abdominal cramps, some vomiting	3-7 or more days	Water or food contaminated with human feces
***E. coli* O157:H7**	Hemorrhagic colitis or *E. coli* O157:H7 infection	1-8 days	Severe (often bloody) diarrhea, abdominal pain and vomiting. Usually, little or no fever is present. More common in children 4 years or younger. Can lead to kidney failure	5-10 days	Undercooked beef (especially hamburger), unpasteurized milk and juice, raw fruits and vegetables (e.g. sprouts), and contaminated water
Hepatitis A	Hepatitis	28 days average (15-50 days)	Diarrhea, dark urine, jaundice, and flu-like symptoms, i.e., fever, headache, nausea, and abdominal pain	Variable, 2 weeks-3 months	Raw produce, contaminated drinking water, uncooked foods and cooked foods that are not reheated after contact with an infected food handler; shellfish from contaminated waters
Listeria monocytogenes	Listeriosis	9-48 hrs for gastro-intestinal symptoms, 2-6 weeks for invasive disease	Fever, muscle aches, and nausea or diarrhea. Pregnant women may have mild flu-like illness, and infection can lead to premature delivery or stillbirth. The elderly or immunocompromised patients may develop bacteremia or meningitis	Variable	Unpasteurized milk, soft cheeses made with unpasteurized milk, ready-to-eat deli meats
Noroviruses	Variously called viral gastroenteritis, winter diarrhea, acute non-bacterial gastroenteritis, food poisoning, and food infection	12-48 hrs	Nausea, vomiting, abdominal cramping, diarrhea, fever, headache. Diarrhea is more prevalent in adults, vomiting more common in children	12-60 hrs	Raw produce, contaminated drinking water, uncooked foods and cooked foods that are not reheated after contact with an infected food handler; shellfish from contaminated waters
Salmonella	Salmonellosis	6-48 hours	Diarrhea, fever, abdominal cramps, vomiting	4-7 days	Eggs, poultry, meat, unpasteurized milk or juice, cheese, contaminated raw fruits and vegetables
Shigella	Shigellosis or Bacillary dysentery	4-7 days	Abdominal cramps, fever, and diarrhea. Stools may contain blood and mucus	24-48 hrs	Raw produce, contaminated drinking water, uncooked foods and cooked foods that are not reheated after contact with an infected food handler
Staphylococcus aureus	Staphylococcal food poisoning	1-6 hours	Sudden onset of severe nausea and vomiting. Abdominal cramps. Diarrhea and fever may be present	24-48 hours	Unrefrigerated or improperly refrigerated meats, potato and egg salads, cream pastries
Vibrio parahaemolyticus	*V. parahaemolyticus* infection	4-96 hours	Watery (occasionally bloody) diarrhea, abdominal cramps, nausea, vomiting, fever	2-5 days	Undercooked or raw seafood, such as shellfish
Vibrio vulnificus	*V. vulnificus* infection	1-7 days	Vomiting, diarrhea, abdominal pain, bloodborne infection. Fever, bleeding within the skin, ulcers requiring surgical removal. Can be fatal to persons with liver disease or weakened immune systems	2-8 days	Undercooked or raw seafood, such as shellfish (especially oysters)

Note. Adapted from *Foodborne Illnesses: What You Need to Know*, by U.S. Food and Drug Administration, 2015.

The more common bacterial foodborne pathogens, such as *Salmonella* species or *Staphylococcus aureus,* remain important; but a number of emerging pathogens, such as *Campylobacter* species, *Calicivirus*, *Cyclospora cayetanensis*, *Listeria monocytogenes*, *Cryptosporidium parvum*, and *E. coli* O157:H7, account for a large proportion of foodborne illness (FDA, 2009). The majority of foodborne illness continues to result from common mistakes in food handling and storing practices in the home or in restaurants. Examples of such mistakes include inadequate cooking temperatures or inappropriate food storage, both of which allow common infectious pathogens such as *Campylobacter* or *Salmonella* species to grow, or other agents such as *S.aureus* to elaborate toxins in food (Kasowski et al., 2002).

Salmonella. *Salmonella* are the most frequently reported bacterial cause of foodborne illness (FDA, 2015). *Salmonella* is a leading cause of illness from food with an estimated 1.4 million cases and 500 deaths per year in the U.S. (Mead et al, 1999). The incidence of salmonellosis appears to be rising both in the U.S. and in other industrialized nations (FDA, 2015). Farmers, industry, food inspectors, retailers, food service workers, and consumers are each critical links in the food safety chain (USDA, 2014).

The *Salmonella* family includes over 2,300 serotypes of bacteria, which are one-celled organisms too small to be seen without a microscope. Two serotypes, *Salmonella* Enteritidis and *Salmonella* Typhimurium are the most common in the U.S. and account for half of all human infections (FSIS, 2014). Strains that cause no symptoms in animals can make people sick, and vice versa. If present in food, it does not usually affect the taste, smell, or appearance of the food. The bacteria live in the intestinal tracts of infected animals and humans (USDA, 2014). Poultry are often implicated as a vehicle of

Salmonella transmission to humans (Bryan & Doyle, 1995). Microbiological surveys indicate that most chickens are not contaminated with *Salmonella* and those that are contaminated usually contain low cells of the organism (Dufrenne et al, 2001). However, *Salmonella* can grow to high numbers on chicken that is subjected to temperature abuse, and even low numbers at processing or at retail can pose significant risk to human health when the product is not properly handled after processing or purchase (Juneja et al, 2007).

Foodservice Packaging

Currently food preservation, quality assurance, and safety are major growing concerns for the food industry. Over time consumers' demand for natural and safe food products with stringent regulations to prevent food-borne infectious diseases is evident (Malhotra, Keshwani, & Kharkwal, 2015). Antimicrobial packaging which is thought to be a subset of active packaging and controlled release packaging is one such promising technology that effectively impregnates the antimicrobial into the food packaging film material and subsequently delivers it over the stipulated period of time to kill pathogenic microorganisms affecting food products thereby increasing the shelf life, (Malhotra et al, 2015).

Polymers for food packaging. The packaging industry has been implementing at a rapidly expanding rate the number of packaging elements made of plastics over recent decades. Plastics, in contrast to more traditional packaging materials like glass and metals, (1) are permeable to the exchange of low molecular weight compounds such as gases and vapors, (2) undergo sorption, so-called scalping, of packaged food constituents,

and (3) are amenable to migration into foodstuffs of packaging constituents (Lagarón, 2011).

The concept of functional or active, bioactive, and intelligent packaging for food applications has recently exploded. An active role has been taken in the preservation, health-promoting capacity and provision of information concerning the products (Lagarón, 2011). Among these factors, active packaging is perhaps the area that has steered more research and industrial interest. Packages may be termed active when it performs some desired role in food preservation other than providing an inert barrier to external conditions. The opportunity of modifying the inner atmosphere of the package or incorporating certain substances in the package wall represents an increasingly productive research area (2011).

CHAPTER III

Methodology

Research Design

For the purpose of this study, time and food product were considered constants. Food product was measured in different environments (fridge, counter, and incubator) under the same time constraints to ensure minimal differentiation in results of bacterial growth. The independent variables consisted of different forms of foodservice packaging, while the bacteria colony count acted as the dependent variable.

Three-level designs. Three-level designs are useful for investigating quadratic effects. The three-level designs are used to model possible curvature in the response function and to handle the case of nominal factors at 3 levels. A third level for a continuous factor facilitates investigation of a quadratic relationship between the response and each of the factors. The three-level design is written as a 3*k* factorial design (NIST, 2012). It means that k factors are considered, each at 3 levels. These are (usually) referred to as low, intermediate and high levels. These levels are numerically expressed as 0, 1, and 2. See Table 3.

Table 3

3^3 Factorial Design

		Factor A		
Factor B	**Factor C**	**0**	**1**	**2**
0	0	000	100	200
0	1	001	101	201
0	2	002	102	202
1	0	010	110	210

Table 3 (cont'd)

1	1	011	111	211
1	2	012	112	212
2	0	020	120	220
2	1	021	121	221
2	2	022	122	222

Note. Adapted from "Three-level full factorial designs," by NIST, 2012, *Engineering Statistics*.

Factor A is the level of bacterial growth (low, moderate, and high respectively), Factor B is the individual foodservice packaging (Styrofoam, plastic and cardboard) and Factor C is the three storage scenarios (counter, fridge, and incubator).

Sample Selection

Due to high unpredictability in the restaurant industry, including but not limited to: storage procedures, various purveyors, and production methods; it is neither feasible nor logical to recreate or test every scenario. In line with attempting to minimize outside variables, food samples were prepared from a singular source. Sysco was selected as the purveyor for the study for apparent consistency in product production across all outlets (SYSCO, 2016). Purchasing from a singular source can warrant more consistent results. Chicken was selected for food sampling because each chicken and its internal organs are inspected for signs of disease by USDA or by State systems that have standards equivalent to the Federal government. The "Inspected for wholesomeness by the U.S. Department of Agriculture" seal ensures the chicken is free from visible signs of disease (USDA, 2014).

Containers. Conducting a comparative study of multiple foodservice packaging, three common types were selected: cardboard, plastic and Styrofoam. To limit outside

variables, all boxes were selected based on equivalent sizes, 5 to 6 inches each, and purchased from a single outlet, Ace Mart Restaurant Supplies. Cardboard foodservice packaging consisted of Envirolines Takeout Box #1, Order #EFTB1N, manufactured in the U.S. from 100% recycled paper and 85% minimum post-consumer content. Plastic foodservice packaging was Dart container 5" Disposable Clear Plastic Hinged To-Go Container, Order #C53PST1. These 5 inch plastic containers from Dart are designed with the ClearSeal® perimeter seal which completely seals the container (ACE MART, 2016). Lastly, the Styrofoam foodservice packaging was Dart Foam Hinged Lid Containers, 6" Large Sandwich Containers, manufactured in the USA, Stock No. 60HT1. All foodservice packing was stored in initial packaging, and sealed.

Media. Among many rapid testing techniques, Petrifilm is considered to be one of the best-known alternatives for enumerating aerobic plate counts (Lakmini & Madhujith, 2012). Petrifilms are ready-to-use products, composed of rehydratable films coated with standard nutrients, a cold water soluble gelling agent, and indicators that facilitate colony enumeration. Petrifilms eliminate the need for preparation of traditional media bringing many advantages over traditional enumeration techniques. For Petrifilm plates, the agar is completely housed in a single unit so that only the sample has to be added, which saves time (Blackburn et al., 1996).

Sample Preparation. Chicken was cooked to an internal temperature of 165°F, and left at room temperature for one hour. This was done to replicate food items purchased, but not fully consumed, during typical dining service. After the allotted time, each food item was distributed evenly and transferred to various foodservice packaging. A total of twenty-seven containers were required, consisting of three cardboard, plastic,

and Styrofoam respectively for each scenario. Cooked chicken from each foodservice packaging and scenario was portioned into 10 g samples in individual stomacher bags, and 90 mL of .1% peptone water was added and pummeled in an AEX Labratore Easy Mix Machine for 120 seconds. Nine milliliters of .1% peptone water was piped into sterile culture tubes, and six dilutions were made by piping 1mL respectively. Petrifilm was placed on a flat surface and 1 ml of sample of each dilution placed at the center of the bottom film by lifting the top film. The top film was carefully replaced and the sample spread using the supplied plastic spreader. The aerobic count Petrifilms were then incubated in a Fisher Scientific Incubator at 37 °C± 1°C for 24 hours and the colonies counted and expressed as the aerobic plate count per gram or per milliliter basis, using a Leica Quebec Darkfield Colony Counter (Blackburn et al., 1996).

Bacterial enumeration. Aerobic plate counts were enumerated over a selected twelve hour timespan; initial count at Hour 0, second count at Hour 6, and a third count at Hour 12. The twelve-hour timespan replicates consumers leaving a food establishment and food product in designated scenarios overnight. The Fisher Scientific Incubator was held at 37°C± 1°C, replicating leftovers overnight in a vehicle; the fridge represented proper storage per HACCP, and the counter was used as an additional variable and potential storage by consumers.

Spread Plating

An additional experiment was conducted to determine the effect of inoculation of chicken with *Salmonella* in various foodservice packaging. Three days prior to each experiment *Salmonella* 53647 was resuscitated by two consecutive transfers to tryptic soy broth and incubated at 37°C± 1°C for 24 hours.

Inoculum Preparation. One milliliter of *Salmonella* was dispensed into 90 mL of .1% peptone water to create a cocktail. Cooked chicken from each foodservice packaging and scenario was portioned into 10 g samples in individual stomacher bags and inoculated with 1mL of the *Salmonella* cocktail. Precaution was taken to dispense as close to the chicken without touching the chicken or stomacher bag itself. The stomacher bag was then carefully held closed and shaken for 30 seconds to assist in distributing evenly, first left to right, then front and back, and clockwise, then counter-clockwise based on the methods established by Neal et al (2008). Inoculated sample bags were then filled with 90 mL of .1% peptone water and stomached for 120 seconds.

Bacterial enumeration. Sigma Aldrich Tryptic Soy Agar was selected as the medium. Approximately 20 mL of melted medium was poured onto sterile petri dishes. Agar was allowed to solidify and petri dishes were brought to and held at room temperature. Nine milliliters of .1% peptone water was piped into sterile culture tubes, and six dilutions were made by piping 1mL respectively. A .1 mL of each of the diluted samples was placed into separate, duplicate, appropriately marked petri dishes. Using a glass spreader held in ethanol, then flamed and allowed to burn off for 15 seconds, the diluted sample was spread staying clear of the edges. After completion of the spread plating, petri dishes were held at 37°C± 1°C for 24 hours.

Data Analysis

Three trials were conducted for each APC for three consecutive trials, based on the "Official Methods of Analysis" by the Association of Official Analytical Chemists. Colony counts were converted to log 10 values and the geometric means were determined. Upon completion of aerobic plate counts and inoculation of *Salmonella*

53647 onto chicken samples, ANOVA was applied to compare counts from the control to that of each sample, to compare the efficacy of each foodservice packaging. Correlations were examined in respect to both environmental scenarios (counter, incubator, refrigerator) to individual food product, and foodservice packaging overall.

CHAPTER IV

Results

The effects of foodservice packaging on microbial growth on temperature-abused leftovers were determined by the ANOVA procedures of SPSS (SPSS, Inc., Chicago, IL). Aerobic plate counts were used to establish a baseline of bacterial growth in comparison to each foodservice packaging, scenario, and hour. Spread plating was used to study the survival and growth of *Salmonella* on chicken in multiple containers, at multiple times, at multiple temperatures.

Aerobic Plate Counts

Data analysis was conducted on 189 variables. Variables included style of packaging (cardboard, plastic, and Styrofoam), multiple scenarios (incubator, counter, and fridge), and time (Hour 0, Hour 6, and Hour 12). Log 10 values of dilution 3 were selected for analysis, because the initial and two progressing dilutions were reported as "Too Numerous to Count" (TNTC). See Tables 4-6.

Table 4

Descriptive Statistics of Foodservice Packaging: Incubator Scenario

	Cardboard (M+/- SD)	Plastic (M+/- SD)	Styrofoam (M+/- SD)
Hour 0	.00 +/- .000	.00+/-.000	.00+/-.000
Hour 6	.58 +/- .786	.03+/-.100	.00+/-.000
Hour 12	.51 +/- .451	.40+/-.801	.61+/-.461

Table 5

Descriptive Statistics of Foodservice Packaging: Counter Scenario

	Cardboard (M+/- SD)	Plastic (M+/- SD)	Styrofoam (M+/- SD)
Hour 0	.00+/-.000	.00+/-.000	.00+/-.000
Hour 6	.00+/-.000	.00+/-.000	.00+/-.000
Hour 12	.00+/-.000	.00+/-.000	.00+/-.000

Table 6

Descriptive Statistics of Foodservice Packaging: Fridge Scenario

	Cardboard (M+/- SD)	Plastic (M+/- SD)	Styrofoam (M+/- SD)
Hour 0	.00+/-.000	.00+/-.000	.00+/-.000
Hour 6	.03+/-.100	.00+/-.000	.00+/-.000
Hour 12	.00+/-.000	.00+/-.000	.00+/-.000

Levene's Test of Equality of Error Variances provided a significance value below .001, thus violating homogeneity of variance. Next, Bonferroni Post Hoc was run showing a significant difference in bacteria growth at hour 0 and hour 12. See Table 7.

Table 7

Bonferroni Post Hoc: Hour

Source	Mean Difference	Standard Error
Hour 0 x Hour 6	-0.07	0.064
Hour 0 x Hour 12	.17*	0.064
Hour 6 x Hour 12	-0.1	0.045

*. The mean difference is significant at the .05 level.

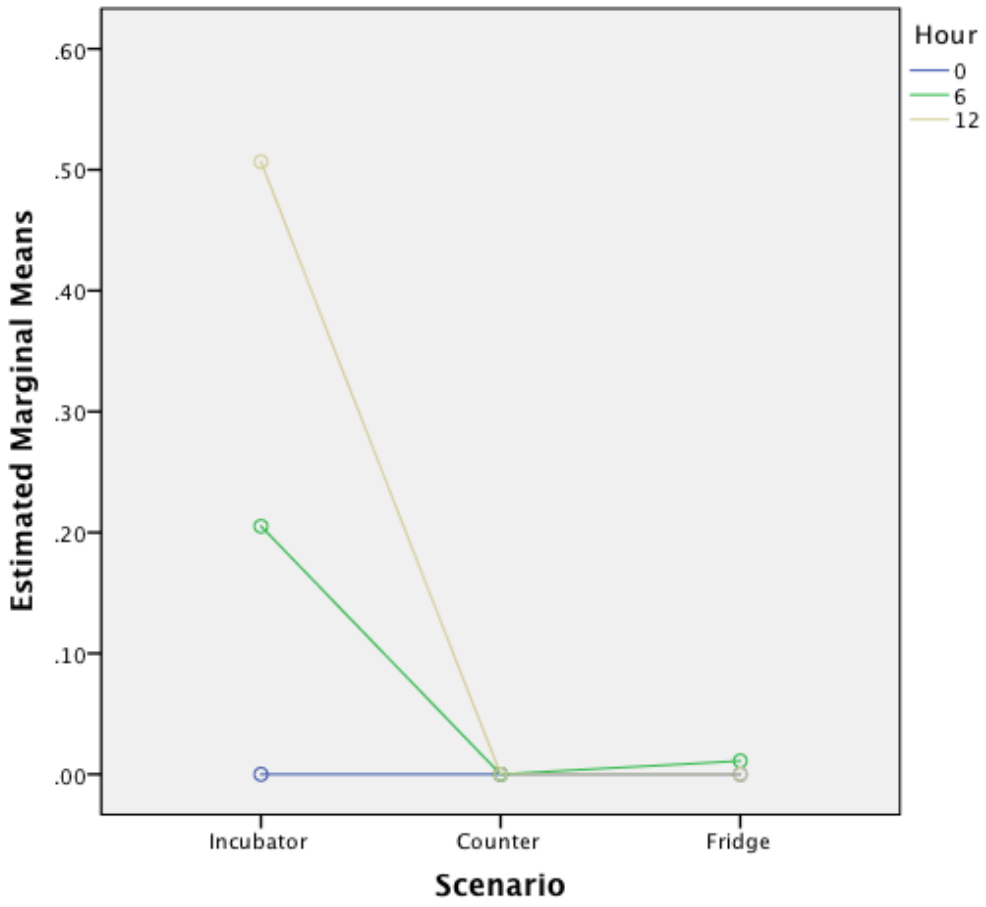

Figure 2. Estimated Marginal Means of LogCFU.

Figure 2 suggests that scenario had a significant difference. While counter and fridge show no significant difference, incubator does suggest significant difference.

Spread Plating

The dependent and independent variables remained the same for both aerobic plate count and spread plating. However, fewer variables were used in the data analysis of *Salmonella*, due to all variables for Hour 12 reported as TNTC ("too numerous to count"). A total of 108 variables were analyzed; 12 variables each for the multiple

foodservice packaging and three different scenarios; 9 variables for hour 0 and 27 variables for hour 6.

Colony counts for *Salmonella* were reported as TNTC for the first four dilutions, thus dilution 5 (log 2.58 CFU/g) was selected. Individual cells in the population are better expressed when a small number rather than a large number of cells are present initially (McKellar & Lu, 2005). Using log 2.58 CFU/g, $p<.05$, proved an unequal variance and violated the assumption of homogeneity of variance. See Table 8.

Table 8

ANOVA Test for Salmonella: Between Packaging, Hour and Scenario

Source	Sum of Squares	*df*	Mean Square	*F*	Significance
Scenario	1.318	2	0.659	1.82	0.191
Packaging	8.592	2	4.296	11.863	0.001*
Hour	4.037	1	4.037	11.147	0.004
Scenario x Packaging	2.179	4	0.545	1.504	0.243
Scenario x Hour	1.571	2	0.785	2.168	0.143
Packaging x Hour	4.702	2	2.351	6.492	0.008
Scenario x Packaging x Hour	3.002	4	0.751	2.072	0.127

*Significant at .001 level.

The ANOVA suggested that packaging had a significant difference. To determine which type of packaging had the greatest significance, a Bonferroni Post Hoc was run. The Post Hoc test revealed that plastic was the most significant, with a significance level below .001. See Table 9 and Figure 3.

Table 9

Bonferroni Post Hoc: Packaging

Source	Mean Diff.	Std. Error
Cardboard x Plastic	1.2033*	0.24568
Cardboard x Styrofoam	0.225	0.24568
Styrofoam x Plastic	0.9783*	0.24568

*. The mean difference is significant at the .05 level.

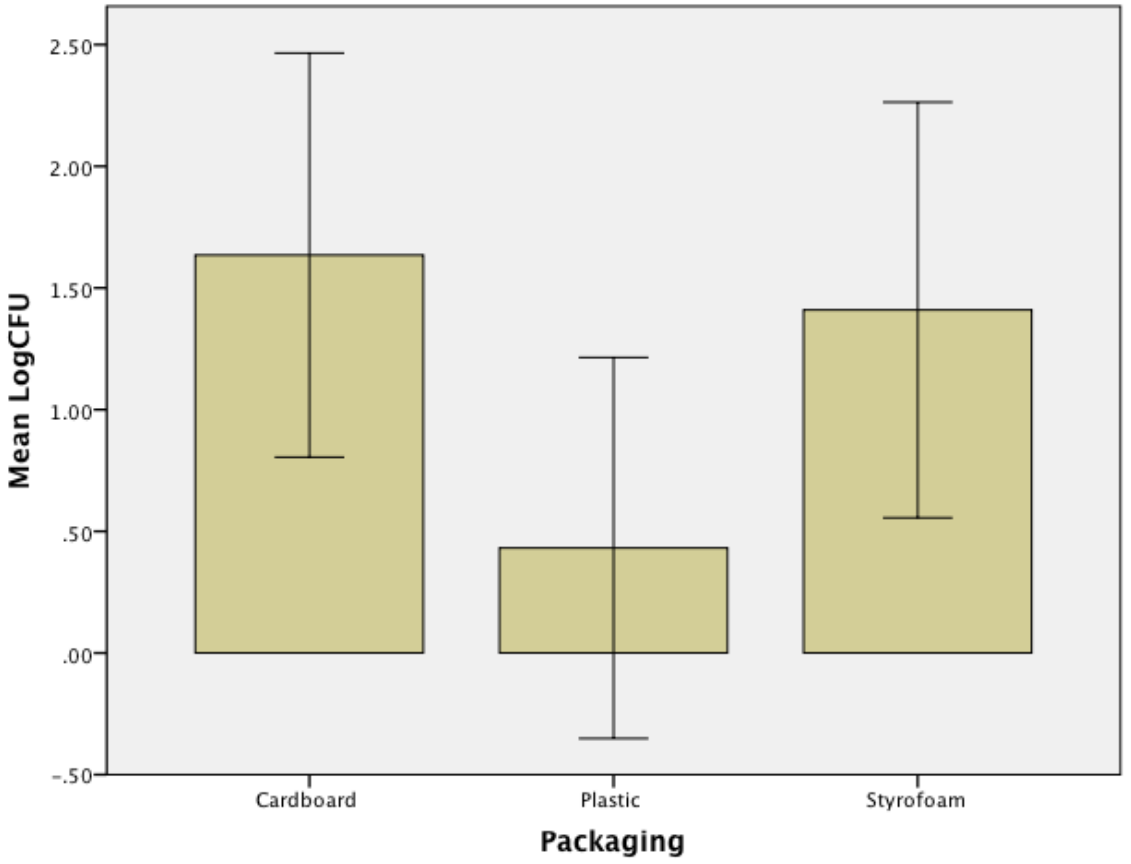

Figure 3. Mean log of colony forming units on packaging. Error bars are +/-1.0 standard deviation.

CHAPTER V

Discussion

The objective of this study was to determine if foodservice packaging could be used as an intervention in preventing microbial growth on leftovers that are temperature abused. Aerobic plate counts were used to establish a baseline of bacterial growth in comparison to each foodservice packaging, scenario, and hour. ANOVA was applied to converted log 10 values of CFUs. Correlations were examined in respect to environmental scenarios (counter, incubator, refrigerator), time (hour 0, hour 6, and hour 12), and foodservice packaging overall (cardboard, plastic, and Styrofoam). An additional experiment was conducted through spread plating, to study the survival and growth of *Salmonella* on chicken in multiple containers, at multiple times, at multiple temperatures.

The results of the initial study suggest that there was no significant difference between multiple packaging in regards to general bacteria growth. Results do conclude though that there was a significant difference in bacteria growth overtime. The findings further insinuate that temperature abuse is a significant risk factor that contributes to greater microbial growth. Leaving food at 37°C, otherwise in a vehicle, proves that bacteria growth significantly increases overtime. The second study examining the survival and growth of *Salmonella* in various packaging, at multiple times and temperatures, concluded that the style of packaging was significant; plastic had the greatest significance, with a significance level below .001. Plastic foodservice packaging had the lowest bacterial counts and may be used as viable tool for reducing microbial populations.

Several studies have found that even when food workers demonstrate knowledge of safe food preparation practices, they do not always engage in said practices (Green & Selman, 2005). Food safety programs must do more than provide food safety training; they should also address the full range of factors that impact food preparation behaviors through implementation and supervision of food safety practices. Reducing the occurrence of foodborne illness risk factors should be a goal for all those involved in food safety. Findings in this study have implications for both industry and consumer. Food safety programs may wish to evaluate and modify their food safety activities by greater emphasizing the need to perform the best practices of cooling hot food to reduce the risk of foodborne illness, and can place reminder stickers on packages for consumers. Efforts should focus not only on how to cool foods properly, but on also why it is important to cool foods properly. Consumers should in turn not temperature abuse leftovers, and be mindful that food safety practices should not only be implemented by foodservice workers, but it is the consumer's responsibility to carry out safe practices of food even thereafter leaving foodservice establishments.

Limitations and Delimitations

A chief limitation to this study was that only selected types of foodservice packaging were tested in the prevention of microbial growth. Different packaging may have concluded that plastic was not the most significant. A second limitation was that the chicken was purchased from a single purveyor, Sysco. While this is typical of most chicken used in restaurants, certain establishments use farm-raised poultry, and the results of this study cannot be generalized to a whole population.

While this study is limited by the aforementioned limitations, it is delimited by only testing restricted amounts of food. Not all food products can be analyzed, and certain untested food items might grow less or more bacteria. Food product restrictions were put in place to control consistent bacterial growth. In addition, not all strains of bacteria could be tested, and different strains may not yield significant variance.

Conclusion

Salmonella bacteria are the most frequently reported cause of foodborne illness. The majority of foodborne illness continues to result from common mistakes in food handling and storing practices in the home and in restaurants. Examples of such mistakes include inadequate cooking temperatures or inappropriate food storage (Kasowski et al., 2002). Shifting the burden of responsibility from the foodservice provider to individual consumers validates the effects of time and temperature abuse. While previous research has indicated that most outbreaks associated with foodservice establishments can be attributed to food workers improper food preparation practices (Green & Selman, 2005), these findings prove that it is not the sole responsibility of restaurant workers.

Though general bacterial growth does not favor one style of foodservice packaging over another, particular strains, specifically *Salmonella*, has a longer survival and greater growth in specific packaging. These results can be useful for guiding future work in analysis of multiple packaging. Future studies may further evaluate if different strains of bacteria will have the same effects when placed under the same or different constraints.

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APPENDIX

Definition of Terms

Aerobic Plate Count: used as an indicator of bacterial populations on a sample. The test is based on an assumption that each cell will form a visible colony when mixed with agar containing the appropriate nutrients. It is not a measure of the entire bacterial population; it is a generic test for organisms that grow aerobically at mesophilic temperatures (25 to 40°C; 77 to 104°F).

Agar Plate Method: Petri dish that contains a growth medium, typically agar plus nutrients, used to culture microorganisms.

Critical Control Point: A point or procedure in a specific food system where loss of control may result in an unacceptable health risk.

Food Safety: Food safety is a scientific discipline describing handling, preparation, and storage of food in ways that prevent foodborne illness.

Foodborne Illness: A disease carried or contracted by eating contaminated food. Usually arises from improper handling, preparation, or food storage.

Foodservice Packaging: Packaging for prepared food items from restaurants or food establishments, used in transporting.

HAACP: Hazard Analysis and Critical Control Point. A systematic approach to identifying, evaluating, and controlling food safety hazards.

Hazard: A biological, chemical, or physical property that may cause a food to be unsafe for human consumption.

Leftovers: Food remaining from a previous meal, remaining as unused portion or amount after the rest has been used or consumed.

Outbreak: Occurs when two or more people become infected from a common food source.

Pathogen: Any disease-producing agent, especially a virus, bacterium, or other microorganism.

Petrifilm: Ready made medium system for enumerating total aerobic bacteria populations. Contain standard methods nutrients, a cold water soluble gelling agent and a tetrazolium indicator dye which facilitates colony enumeration.

Restaurant: Establishment that prepares and serves food or beverages to customers, but is not an institution, food cart, mobile food unit, temporary food stand, supermarket, or caterer.

Temperature Danger Zone: 40°F to 135°F, the range in which pathogenic bacteria can multiply rapidly in a food and possibly cause foodborne disease.

Time Temperature Abuse: Occurs when food is not cooled properly, not stored or held at required temperatures, or allowed to remain in a temperature that is favorable to the growth of microorganisms.