ABSTRACT

THOMAS, ELLEN MARIE. Tracing Temperature Patterns of Cut Leafy Greens during Transportation and Service. (Under the direction of Benjamin Chapman).

Leafy greens have been linked to over 40 outbreaks of foodborne illness since 1990. As potential pathogens such as *Escherichia coli* O157:H7 can grow on or in cut leafy greens at temperatures above 5°C, the U.S. Food and Drug Administration recommends that leafy greens be kept at refrigerated temperatures (5°C or lower).

There is a lack of research in determining the temperature profiles of leafy greens within food service establishments, especially the variation in temperatures through storage, preparation, serving, restorage, and reservice. The purpose of this study was to determine the temperature patterns that leafy greens in single serving clamshell containers were exposed to through a typical school meal program.

Temperatures of ready-to-eat leafy greens in clamshell containers were recorded using data loggers in 24 schools over a 3-day period. Temperatures were taken by stabbing the probe into the leafy greens in the center of the container.
Various temperature patterns were seen, including temperatures rising above 5°C for at least one hour up to 3 consecutive days. In some cases, temperatures reached above 5°C for more than 3 hours throughout serving time. Leafy greens were shown in all cases to be exposed to temperature variability.

The second portion of this study consisted of addressing the concerns raised by farmers in North Carolina about the new requirement that leafy greens must be received at establishments no higher than 5°C. Leafy greens were held in different types of coolers with ice and ice packs over four hours in a car trunk (to mimic delivery conditions). Three coolers were used: a large Coleman, a small Igloo, and a Styrofoam cooler. Temperatures of leafy greens were recorded using data loggers by stabbing the probe directly into the bagged leafy greens; multiple data loggers were used in each cooler.

There was an increase in temperature over the four hours in all coolers with ice packs. Leafy greens experienced a decrease in temperature when held in the large and small Igloos with ice. These results show that some methods of holding leafy greens are effective at keeping leafy greens below 5°C for at least four hours.

The results show the importance of developing a standard for temperature monitoring of leafy greens during transportation, storage, and service to avoid the potential for harmful pathogen growth. This data can be used both in risk assessment calculations and as an example of the need for consistent policy in institutional leafy green holding practices.

Tracing Temperature Patterns of Cut Leafy Greens during Transportation and Service

by
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BIOGRAPHY

Ellen grew up on a farm in Fryburg, Pennsylvania and has always been interested in the agricultural sciences. She looks forward to a challenging and exciting career in food safety.

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I began graduate school excited about the prospects of a food science career, but I had little idea of the vast possibilities for research projects. Then, I met Allison Smathers at a Food Science Club event where she told me about her food safety research with Dr. Ben Chapman. After my first meeting with Ben, I was even more intrigued by the world of food safety communication and I was excited to join his group.

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CHAPTER 1

INTRODUCTION

The shift towards healthier eating, the desire for easily accessible and prepared food, and the ability to transport food long distances have all contributed to an increase in pre-packaged, cut leafy greens sold in grocery stores, served in restaurants and institutional kitchens, and consumed at home.

An increase in foodborne illness associated with leafy greens has accompanied the increase in leafy green consumption. The act of cutting the leaf exposes plant tissue, which serves as nutrients for bacteria. Further, there is no kill step involved in the preparation of fresh leafy greens, which make cut leafy greens a high-risk product.

A great deal of research has been conducted to pinpoint the various methods by which microbial contamination of fresh produce can occur in the field, during harvest and processing, and during preparation. Temperature can contribute to further pathogen proliferation. As a result, the U.S. Food and Drug Administration (FDA) Food Code sets certain temperature regulations to ensure safe transportation and storage conditions of leafy greens. In order to adhere to this regulation, the industry has implemented various policies and systems for transporting and storing leafy greens. However, there is a gap in the literature surrounding these policies; little work has been done to determine whether these are the best methods in place for reducing the risk associated with consuming leafy greens.

This study monitored temperatures of leafy greens during transportation and during serving in order to fill the gaps in research with real data. The focus of this project was to determine whether current policies are the most effective methods to manage risk.

CHAPTER 2
LITERATURE REVIEW

2.1 Introduction

It is estimated that foodborne pathogens cause 48 million illnesses of known etiology in the U.S. annually including 130,000 hospitalizations and 3,000 deaths (Scallan et al., 2011). Estimates suggest that foodborne viruses make up 58 percent of these illnesses, while bacteria cause 39 percent (Scallan et al., 2011). Bacterial pathogens can be associated with a wide variety of foods, including meat, seafood, canned foods, baked goods, and produce. Unlike viruses, which are not living, bacteria favor certain growth conditions such as high pH, an environment with a high water activity and plenty of nutrients, and a temperature near 37°C. These factors must be controlled in order to reduce risk of pathogen growth.

Recently, foodborne outbreaks associated with produce have increased (Palumbo et al., 2007). An outbreak is defined as a situation where two or more people become ill from eating a common food (CDC, 2000). From 1996 to 2008, approximately 35 percent of produce outbreaks were associated with leafy greens (FDA, 2009). The 2009 U.S. Food and Drug Administration (FDA) model Food Code defines leafy greens as lettuce, spinach, spring mix, cabbage, arugula, kale; it does not include herbs (FDA, 2009). This definition establishes which products would be classified as requiring time/temperature control for safety (TCS) in the event of being cut (FDA, 2009). It is believed that the increase in outbreaks associated with leafy greens can be attributed partially to the increase in sales of bagged product in the past 15 years (Palumbo et al., 2007).

2.2 Outbreaks

Since 1995, there have been 53 reported outbreaks associated with leafy greens (CDC, 2011). Implicated pathogens include norovirus, *Salmonella* spp, *Campylobacter*

jejuni, and *Shigella sonnei*, but the majority of the outbreaks (35) were caused by *Escherichia coli (E. coli)* serotypes, primarily *E. coli* O157:H7 (CDC, 2011).

Pathogenic *E. coli* can be extremely harmful due to their production of shiga toxin; these *E. coli* are known as shiga toxin-producing *E. coli* (STEC) (CDC, 2011). The mean infectious dose for STEC is very low (CDC, 2011). STEC infections can cause abdominal cramps, vomiting, and bloody diarrhea and lasts approximately five to seven days; in very serious cases, hemolytic uremic syndrome (HUS) can occur (CDC, 2011). STEC can be particularly dangerous for young children, the elderly, pregnant women, and the 3mmune-compromised (CDC, 2011).

The first reported outbreak of *E. coli* O157:H7 associated with the consumption of cut lettuce occurred in 1995. Cut leafy greens are defined as fresh leafy greens that have been cut, chopped, shredded, sliced, or torn; it does not include leafy greens cut from the root in the field without any further processing (FDA, 2009). Forty Montana residents tested positive for the pathogen after purchasing the lettuce at local grocery stores (Ackers et al., 1998). To trace the source of an outbreak, public health officials conducted case-control studies, which consist of interviews of patients and controls to establish food consumed, symptoms, and other food handling practices (Ackers et al., 1998). Stool and serum samples were collected from patients showing symptoms of diarrhea and abdominal cramps; investigators identified the strain through pulsed-field gel electrophoresis (Ackers et al., 1998). The strain was linked to cut lettuce from both a farm in Montana and six farms in Washington shipping within the same label (Ackers et al., 1998). Processing did not include washing before packing and delivery; lettuce was harvested, packed in boxes, and delivered (Ackers et al., 1998). An environmental investigation was conducted, which included collection of leaf samples in the field, irrigation water, fecal samples, and compost, as well as an examination of growing, harvesting, and handling processes on the farm, transportation procedures, and handling practices at the retail level (Ackers et al., 1998). It is unclear exactly how contamination in this case occurred. Investigators did outline four possibilities: fertilization of the lettuce with

cattle manure that may not have been treated properly; contamination by feces through irrigation or water runoff; direct feces contamination as cattle had access to the stream used for irrigation; or, contamination of the irrigation water or lettuce by sheep or deer (Ackers et al., 1998). Due to the fact that *E. coli* O157:H7's mean infectious dose is low, it is likely that the initial contamination level was small and that cross-contamination between different batches of lettuce occurred (Ackers et al., 1998). Temperature during transportation and storage may have also played a role in bacterial proliferation. Jol and colleagues (2005) found that approximately 20% of domestic and commercial refrigerators within the United States operate above 10°C; numerous studies have shown that *E. coli* O157:H7 can grow at temperatures below 12°C (Abdul-Raouf et al., 1993; Ackers et al., 1998; Khalil and Frank, 2010; Lopez-Velasco et al., 2010; Luo et al., 2009). Although the exact source of contamination is unknown, numerous factors may have contributed to making the outbreak larger; this was the case in several subsequent outbreaks. Numerous pathogenic *E. coli* outbreaks linked to leafy greens have occurred in the United States since (Powell, 2008). For a cited list of other *E. coli* outbreaks associated with leafy greens in the United States and Canada, see Table 2.1.

Table 2.1. Referenced *E. coli* outbreaks associated with leafy greens in the United States and Canada from 1995 to 2011.

Year	Pathogen	Number infected	Leafy green implicated	Processing	Location	Citation
October 2011	*E. coli* O157:H7	60	Romaine lettuce	Cut; prewashed (salad bar)	Multistate	CDC, 2011
May 2010	*E. coli* O145	26	Romaine lettuce	Cut; prewashed (salad bar)	Illinois	CDC, 2010
November 2008	*E. coli* O157:H7	130	Lettuce	Unknown	Ontario, Canada	Entis, 2008

Table 2.1 Continued

September October 2008	*E. coli* O157:H7	59	Lettuce	Cut/bagged ; foodservice	Multistate and Canada	CDPH, 2010
May/June 2008	*E. coli* O157:H7	9	Lettuce	Unknown	Washington	Clark, 2008
November 2006	*E. coli* O157:H7	77	Lettuce	Cut, prewashed	Multistate	CDC, 2006
October 2006	*E. coli* O157:H7	205 (3 deaths)	Spinach	Cut; prewashed	Multistate	CDC, 2008
September 2005	*E. coli* O157:H7	31	Lettuce	Cut, prewashed	Multistate	*CDPH, 2005*
November 2001	*E. coli* O157:H7	20	Lettuce	Unknown	Texas	Smith Dewaal et al., 2006
October 2000	*E. coli* O157:H7	6	Salad	Unknown	Indiana	Smith Dewaal et al., 2006
October 1999	*E. coli* O157:H7	40	Lettuce	Unknown	Pennsylvania	Smith Dewaal et al., 2006
October 1999	*E. coli* O157:H7	47	Lettuce	Unknown	Ohio, Indiana	Smith Dewaal et al., 2006
October 1999	*E. coli* O157:H7	5	Salad	Unknown	Oregon	Smith Dewaal et al., 2006
September 1999	*E. coli* O111:H8	58	Lettuce	Unknown	Washington	Smith Dewaal et al., 2006

Table 2.1 Continued

February 1999	*E. coli* O157:H9	65	Lettuce	Unknown	Nebraska	Smith Dewaal et al., 2006
June 1996	*E. coli* O153:H49	7	Lettuce	Unknown	New York	Smith Dewaal et al., 2006
October 1995	*E. coli* O153:H46	11	Lettuce	Unknown	Ohio	Smith Dewaal et al., 2006
September 1995	*E. coli* O153:H47	30	Lettuce	Unknown	Maine	Smith Dewaal et al., 2006
September 1995	*E. coli* O157:H7	20	Salad	Unknown	Idaho	Smith Dewaal et al., 2006
July 1995	*E. coli* O153:H48	74	Lettuce	Cut; unwashed	Montana	Smith Dewaal et al., 2006

Several *E. coli* O157:H7 outbreaks associated with lettuce and spinach occurred in 2008 (Powell, 2008). Three small outbreaks occurred early in 2008 in Oregon, Washington, and California, sickening five to nine people in each case (Powell, 2008). Thirty-four illnesses were reported and 18 people were hospitalized in an outbreak associated with iceberg lettuce in Michigan (Powell, 2008). Product traceback and the Michigan Department of Agriculture pinpointed contaminated lettuce to one local wholesale distributor of bagged, industrial-sized packaged iceberg lettuce in Detroit, which was supplied by California (Powell, 2008). These outbreaks differ from the 1998 *E. coli* O157:H7 outbreak because it was a processed product, showing that processed product can be contaminated as well. The

FDA defines processed leafy greens as those that have been cut, undergone a series of washes, and bagged (FDA, 2009).

In the spring of 2010, an outbreak of *E. coli* O145 associated with shredded romaine lettuce served in retail salad bars sickened over 50 people (CDC, 2010). Shredded lettuce from one packaging facility was implicated, which was harvested from one single farm (CDC, 2010). In late 2011, another outbreak associated with Romaine lettuce served in salad bars sickened 60 people in 10 states (CDC, 2011). Traceback investigations revealed that romaine lettuce was contaminated from one lot on one farm (CDC, 2011).

Temperature abuse during storage has also contributed to outbreaks where initial contamination levels were low. Although *E. coli*'s optimal growth temperature is approximately 37°C, it can still grow slowly at lower temperatures. Its growth rate increases as the environmental temperature approaches the optimum.

In 2006, spinach and lettuce contaminated with *E. coli* O157:H7 contributed to 375 illnesses, 192 hospitalizations, approximately 40 cases of hemolytic uremic syndrome (HUS), and three deaths in three different outbreaks (CDC, 2011). The most notable of these 2006 outbreaks occurred in September; bagged spinach sickened 206 people and killed three. Initially, cattle fecal contamination was suspected, but molecular data also revealed contaminated samples from feral swine and a nearby stream (California Food Emergency Response Team, 2007; Grant et al., 2008; Jay et al., 2007). Subsequent reports of the outbreaks support the fact that good growth conditions (in this case, temperature) allowed for an increase in *E. coli* counts when initial contamination levels were low (CDHS, 2007; Danyluk and Schaffner, 2011).

Determining the site of contamination is difficult for several reasons. First, there are multiple sites through which contamination could occur, including in the field, during processing, and during preparation if coming into contact with other product (Beuchat, 1998). Investigations do not often determine the amount of contamination, even if the contamination source has been pinpointed. Despite advanced techniques for pinpointing a

particular strain of pathogen, there is no certain way of knowing how many bacteria were initially present. Further, the mean infectious dose for *E. coli* O157:H7 is so low that the presence of any of this pathogen is a problem. Investigations are limited by the fact that *E. coli* O157:H7 is typically present at the contamination site in low concentrations. Processing steps, including mixing of multiple batches, washing, and bagging of product are all relatively temperature controlled, making it unlikely that bacterial populations could proliferate at high levels. It is more likely for bacteria to proliferate at high levels when temperature increases. Even when good epidemiological techniques are used, it is unlikely that the actual pathogen will be found; the 2006 outbreak investigation results were unusual in that the pathogen was actually detected.

2.3 Contamination Factors

There are many opportunities for pathogens to be introduced to foods along the farm-to-fork continuum. Contamination in the field can occur from soil, water, wild and domestic animals, equipment, and staff (Beuchat, 1998; Johnston et al., 2005). Many pathogens are persistent in soil and have long-term viability (Beuchat and Ryu, 1997). Improperly treated irrigation water or irrigation water that contains raw sewage can carry a wide variety of pathogens, including norovirus, hepatitis A viruses, *E. coli* O157:H7, *Salmonella* species, and *Shigella* species (Beuchat, 1998). *E. coli* O157:H7 can be transmitted through both drip and spray irrigation, and is able to survive on leaf surfaces for long periods of time (Khalil and Frank, 2010; Solomon et al., 2002). Numerous outbreaks have been associated with fecal contamination by both domestic and wild animals (Brackett, 1999).

There are also multiple vectors for contamination at the post-harvest level. Contamination of wash water can occur through produce already contaminated or through workers practicing poor hygiene (Suslow, 1997). Several multistate outbreaks have been traced to one or two batches of contaminated product that cross-contaminated other lettuce when several batches were mixed together. Infrequent or poor sanitation of transporting and

processing equipment can also contribute to contamination (Johnston et al., 2005). Food handlers at home and in commercial kitchens also have the potential to introduce pathogens (Doering et al., 2009).

Approximately 59 percent of foodborne illnesses can be traced to food served outside the home (CDC, 2006). Another outbreak study of 25 European countries estimate that approximately 40 percent originate in the home, 20 percent are linked with restaurants, and the remaining 40 percent are associated with meals consumed in schools, hospitals, and other institutional kitchens (Tirado and Schmidt, 2000). Pinpointing where the contaminated food was consumed is required in order to control an outbreak, but investigating where contamination occurs and the risk factors involved is necessary to understanding how best to reduce foodborne illness (Jacob and Powell, 2009).

The top risk factors established by the 2009 U.S. Food and Drug Administrations (FDA) Model Food Code are improper holding temperatures, inadequate cooking procedures, contaminated equipment, food from unsafe sources, and poor personal hygiene (FDA, 2009). An FDA study (2001) examined data collected during inspections of the following types of kitchens: institutions (schools, nursing homes, and hospitals), restaurants (fast food and full-service), and retail food stores (FDA, 2001). The 1997 Food Code was used as the reference point for classifying violations. This included data on food sources, records, cooking temperature, hot holding, cooling procedures, treatment of food contact surfaces, handwashing, good hygienic practices, and use of chemicals. A data collection form, modeled on the factors, was used to designate whether a facility was considered in compliance or out of compliance based on standards stipulated directly in the Food Code (FDA, 2001). Up to 55 sites were observed for each data item. Based on violations designated by the Food Code, the data showed that only about 60 percent of quick-service and 74 percent of full-service restaurants complied with standards (FDA, 2001). Retail departments' compliance rates for deli, meat and poultry, produce, and seafood were 73 percent, 81 percent, 76 percent, and 83 percent, respectively. Hospitals, nursing homes, and

elementary schools had compliance rates of 80 percent, 82 percent, and 80 percent, respectively (FDA, 2001). Of the institutional kitchens (schools, nursing homes, and hospitals), cross-contamination was the highest occurring risk factor at a rate of 70 percent, followed by improper holding time/temperature (67 percent), and poor personal hygiene (55 percent) (FDA, 2001). An overall understanding of how fast-paced kitchens run, and tracing where the violations originate, are crucial to controlling critical factors in food handling.

2.4 Observation Studies

Direct observation of food handling is a necessary component to understanding where risky practices are occurring. Kennedy and colleagues (2011) used marketing consultants to recruit participants (n=60) representative of typical food shoppers and consumers in Dublin, Ireland. Researchers observed the participants preparing a meal at home, sampled potential sites for contamination by *Campylobacter jejuni*, *Staphylococcus aureus* and *E. coli* species throughout the kitchen, and administered a survey in order to gauge knowledge of food safety practices (Kennedy et al., 2011). Researchers asked participants about how they viewed the importance of various food safety practices (Kennedy et al., 2011). Scores were developed based on the microbiological test results, hand cleanliness, and an observation checklist (Kennedy et al., 2011). Refrigerator handles, sink drains, sink faucets, work surfaces, knives, and cutting boards were swabbed for pathogen testing (Kennedy et al., 2011). Swabs were also taken of food handlers' hands, in addition to samples from the cooked food products (Kennedy et al., 2011). The three main "critical points" were correct cooking practices, prevention of cross-contamination, and correct food storage practices (Kennedy et al., 2011). These scores showed a low level of food safety knowledge correlated with unsafe food handling behavior (Kennedy et al., 2011).

Roberts and colleagues (2008) conducted a study in which food handling behaviors of participants were observed, after which food safety training was administered to participants. This was followed by more observation in order to compare compliance and knowledge rates

before and after training. A written knowledge assessment was administered to 160 employees both before and after the food safety training and was composed of questions pertaining to 3 behaviors: cross contamination, time and temperature abuse, and personal hygiene (Roberts et al., 2008). Despite the fact that knowledge scores increased significantly between the pre-training and post-training assessments, handwashing was the only category in which there was a significant improvement from pre- to post-training (Roberts et al., 2008). Behavioral compliance remained low (below 30 per cent compliance in some cases) for individual behavior categories even after food safety training (Roberts et al., 2008). There was little impact on cross-contamination and thermometer use (Roberts et al., 2008). The handwashing training portion included a hands-on activity, which may account for the improvement; also, employees demonstrated the most previous knowledge about the importance of handwashing compared to the other behaviors (Roberts et al., 2008). The data from Kennedy and Roberts show that adherence to safe food handling behaviors in kitchen settings is low, even after food safety training. High knowledge scores did not equate to high behavior scores because knowledge is not enough to change behavior. Not only is knowledge important, but understanding the "why" of food handling is crucial to behavior change as well (Roberts et al., 2008). Chapman and colleagues (2010) provided food handlers with printed graphic examples in which foodborne illnesses resulted from poor food handling practices. Food handler behavior was observed after being introduced to food safety infosheets; there was a significant increase in handwashing attempts and a significant decrease in cross-contamination situations (Chapman et al., 2010). Regardless of where contamination of a product occurs, subsequent temperature abuse of the product will result in an increased risk for foodborne illness.

Risk management is a field that consists of identifying risk of a product or process and then taking steps to reduce that risk (Buchanan, 2011). Hazard Analysis of Critical Control Points (HACCP) is an example of risk management, which consists of identifying opportunities where contamination may occur, and implementing steps to reduce the amount

of contamination, or preventing it altogether. Temperature control plays a crucial part in the risk management of cut leafy greens. If the product has been contaminated, this temperature control step serves as a risk reduction strategy by preventing further proliferation of a pathogen.

2.5 Storage Temperature

FDA recommends that fresh-cut leafy greens be kept at 5°C or lower (FDA, 2009). Several studies have examined the effect of varying storage temperatures of leafy greens on *E. coli* O157:H7 growth (Table 2.2). Luo and colleagues (2009) inoculated spinach leaves with *E. coli* O157:H7 and exposed them to a range of temperatures over several days in order to observe growth levels. Leaves kept at temperatures deemed "abusive" (8°C or above) supported a 1-2 log growth within 3-6 days, even before the product underwent significant product degradation (Table 2.2) (Luo et al., 2009). Abdul-Raouf and colleagues (1993) measured the growth of 5 strains of *E. coli* O157:H7 on iceberg lettuce at storage temperatures of 5°, 12°, and 21°C for 14 days. Populations decreased at 5°C, and increased at 12° and 21°C (Table 2.2) (Abdul-Raouf et al., 1993). Khalil and Frank measured growth of *E. coli* O157:H7 on different leafy greens (baby Romaine lettuce, cilantro, spinach, and parsley). Leaves were inoculated with approximately 10^5 CFU/ml of the pathogen and held at different "abusive" temperatures (8° and 12°C). *E. coli* populations grew on spinach leaves held at 8°C and 12°C after 3 days (Khalil and Frank, 2010). Growth of *E. coli* O157:H7 did not occur on romaine lettuce at 8°C or 12°C, but did occur at 15°C after 8 hours (Khalil and Frank, 2010). *E. coli* growth also did not occur at 8°C for 4 days on cilantro or parsley (Table 2.2) (Khalil and Frank, 2010). These results suggest that storage temperature plays an important role in the ability of bacteria to grow on leafy greens, and that spinach and lettuce may be especially sensitive to the increase in temperature.

Lopez-Velasco and colleagues (2010) compared the growth of *E. coli* O157:H7 held at normal retail storage temperatures of 4°C and 10°C over 15 days to that of phylloepiphytic

bacteria (typical background microorganisms adapted to grow on plant leaves). Fresh spinach and commercial spinach were held at 4°C and 10°C until spoilage. By day 15, there was a 5-log fold increase in *E. coli* O157:H7 populations at 10°C and a 3.6-log fold increase at 4°C (Lopez-Velasco et al., 2010). Of the spinach held at 4°C, *E. coli* levels dropped initially between days 0 and 5, but by day 15 had increased again, suggesting that *E. coli* O157:H7 can adapt to low temperatures (Lopez-Velasco et al., 2010). The expression of 2 attachment genes required for bacteria establishment on a surface, espA and csgA, increased at 10°C; these attachment genes are associated with *E. coli* O157:H7 virulence (Lopez-Velasco et al., 2010). Expression still occurred at 4°C, but at a much lower level than at 10°C (Table 2.2) (Lopez-Velasco et al., 2010).

Li and colleagues (2001) measured the effect of temperature abuse and treatment with chlorine on *E. coli* O157:H7 survival and growth on iceberg lettuce stored at 5°C for 18 days and at 15°C for 7 days. Lettuce was inoculated both before and after heat and chlorine treatment. *E. coli* viability decreased over the 18 days at 5°C (Li et al., 2001). At 15°C, the population increased by nearly 1 log over 2 days, then increased more slowly over the remaining 5 days of storage (Table 2.2) (Li et al., 2001). Li and colleagues demonstrated that even with a pathogen-reduction step, temperature is still an important control point in keeping bacterial counts low (Li et al., 2001).

Palumbo and colleagues (2007) discuss current FDA recommendations for handling and storing of fresh-cut leafy greens based on recent outbreaks, as well as current research about bacterial growth conditions. The directions for properly chilling food read, “Store RTE lettuce/leafy green salads in the refrigerator” (Palumbo et al., 2007; FDA 2001). However, there is no actual definition of refrigeration within the documents, nor are there clear definitions about an acceptable time frame during which the product temperature could exceed refrigerated temperature, such as during serving (Palumbo et al., 2007; FDA 2001).

Table 2.2. Storage temperature studies of *E. coli* O157:H7 on leafy greens with temperature and growth rate.

Study	Temperature	Growth rate
Luo, et al.	8°C	1-2 log (3 days), Continued to grown slowly
Abdul-Raouf, et al.	5°C 12°C 21°C	Decrease 1-2 log (3-7 days) 3-4 log (3-7 days)
Khalil and Frank	8°C 12°C	1.18 log 2.08 log
Lopez-Velasco, et al.	4°C 10°C	3.6 times (15 days) 5 times
Li, et al.	5°C 15°C	Decrease 2-3 log (2 days), Continued to grow slowly

2.6 Modeling Conditions

Doering and colleagues (2009) examined the effect of resident microflora on the ability of *E. coli* O157:H7 to grow on lettuce and spinach. This included the lag time between harvesting and processing, transportation conditions, washing process, and holding time before and after cooling (Doering et al., 2009). The lowest field temperature and shortest waiting period negatively affected *E. coli* populations the most (Doering et al., 2009). Field temperature and transportation temperature were less important factors once the storage period began (Doering et al., 2009). At 25°C, *E. coli* increased over five days (Doering et al., 2009). At 32°, the bacterial population increased rapidly by day 2 and at this point the product was deemed "spoiled" (Doering et al., 2009). Overall, the lower field temperature and lowest storage temperature combined resulted in the lowest psychrotrophic populations (Doering et al., 2009).

Danyluk and Schaffner (2011) developed a quantitative risk model based on existing data regarding different variables' effects on *E. coli* O157:H7 growth, including retail storage temperatures, and home storage times and temperatures. The authors used linear regression to model the relationship between temperature and growth rate, and compared this model to the

USDA-ARS Pathogen Modeling Program and another model of bacterial growth on iceberg lettuce developed by Koseki and Usobe (Danyluk & Schaffner, 2011). Based on their model, the output predicted that, in situations of temperature abuse, *E. coli* O157:H7 growth on cut leafy greens would be supported, and could occur at a rate of up to 1 log CFU/day (Danyluk & Schaffner, 2011).

2.7 Conclusions

Outbreaks associated with cut leafy greens have increased within the past fifteen years. There are multiple pathways for pathogens to be introduced along the continuum from the field to the consumer. Similarly, numerous factors can contribute to proliferation of harmful bacteria once contamination has occurred; one of these factors is temperature. Numerous studies have demonstrated the ability of pathogens, particularly *E. coli* O157:H7, to grow at storage temperatures above 5°C.

Temperature has played a significant role in contributing to outbreaks associated with *E. coli* O157:H7 and cut leafy greens. If contamination has occurred earlier in the chain, temperature abuse during storage or service of the product can aid in the proliferation of pathogens. This data indicates the need for information on storage temperatures of leafy greens in order to evaluate this particular risk factor.

Temperature control of cut leafy greens is a crucial step when working to manage risk of foodborne illness. Research has been done to examine the effect of various temperature patterns on bacterial growth, but very little information has been collected in reference to the temperatures actually occurring during transportation and service. Collecting this temperature information is crucial in determining whether current policies for proper storing and serving temperatures for leafy greens are effective in preventing growth of pathogens.

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CHAPTER 3
TRACING TEMPERATURE PATTERNS OF CUT LEAFY GREENS IN INSTITUTIONAL KITCHENS

3.1 Introduction

Improper holding temperature is one factor that contributes to foodborne illness (FDA, 2009). Temperature abuse during storage has contributed to foodborne outbreaks where initial contamination levels were low (CDC, 2011). Leafy greens were added to the 2009 FDA Food Code as a food that requires time and temperature control (FDA, 2010). This new designation occurred because the act of cutting the leaf exposes plant tissue, providing nutrients for bacteria and allowing for growth of pathogens when kept at temperatures above 41°F (FDA, 2010). There have been 53 reported outbreaks throughout the United States associated with leafy greens since 1995 (CDC, 2011). These outbreaks occurred as a result of contamination in the field or during processing; other factors such as temperature abuse and cross-contamination also contributed to these outbreaks after initial contamination. *E. coli* O157:H7 is the primary pathogen of concern, as it is able to attach to cut portions of the leaf (Takeuchi and Frank, 2000). It also has a very low mean infectious dose. FDA recommends that fresh-cut leafy greens be kept at 5°C or lower due to research showing that pathogens such as *E. coli* O157:H7 are capable of growing at temperatures above 5°C (Abdul-Raouf et al., 1993; FDA, 2009; Khalil and Frank, 2010; Li et al., 2001; Lopez-Velasco et al., 2010; Luo et al., 2009). If contamination has occurred prior to storage at the facility, holding the leafy greens at or below 5°C will prevent further growth of the pathogen (FDA, 2010).

According to the Federal Register (74 FR 66213), the USDA requires that all schools must have a food safety plan based on Hazard Analysis Critical Control Points (HACCP) (USDA, 2005). HACCP is designed by identifying hazards within a process, pinpointing critical control points that can be implemented to control these hazards, and determining

methods for monitoring and taking corrective steps when needed (USDA, 2005). USDA began requiring a HACCP plan because HACCP examines each step of the food preparation process in order to reduce risk, but still allows for flexibility within the lunch program (USDA, 2005). North Carolina School HACCP is based on the FDA Food Code, and so the addition of cut leafy greens to the 2009 Food Code is now reflected in North Carolina School HACCP guidance. To support the School HACCP program, North Carolina's Department of Public Instruction has a food safety team made up of representatives from each school district to provide guidance for school lunch programs and to evaluate compliance. This includes the monitoring of program standards, food handling practices, food storage records, and the training of food handlers. The NC School HACCP program also recommends food safety training for all food handlers.

Delivery of leafy greens to school kitchens in North Carolina occurs once a week. North Carolina schools purchase leafy greens from both private food service companies and the farm to school program, which is administered by the North Carolina Department of Agriculture and Community Supported Agriculture (CSA) in order to purchase food from local farms. Most schools order both whole head lettuce and shredded lettuce; the amount is dependent on the menu and amount of food to be prepared.

Upon delivery, lettuce is brought into a large walk-in storage cooler, where it is held until it is prepared. Once a new shipment of leafy greens is received, any leafy greens remaining in the cooler are discarded. Other food products requiring refrigeration, such as fruit, are also stored in the walk-in cooler. An employee records the storage cooler temperature at least once per day on a temperature log. The temperature logs are attached to the wall adjacent to the cooler. Storage coolers are held between 2° and 5°C.

Typical preparation of salads includes washing, then weighing and placing in clamshell containers. If a package of leafy greens is labeled "pre-washed," further washing is not required. *E. coli* O157:H7 adheres to the cut portion of the leaf and can remain attached even after a chlorine wash treatment, which suggests that further washing will not remove the

bacteria (Palumbo et al., 2007). Additional washing by the food handler is not likely to improve the safety of the product; in fact, further washing may lead to cross-contamination, outweighing the benefit of washing (Palumbo et al., 2007; Seo and Frank, 1999). Other toppings specified by USDA meal guidelines are added after any washing steps. There is some variation in preparation steps among schools based on the number of salads to be prepared; this variation is primarily applied to the amount of time required to make the salads. Depending on the school's serving policy, salads are either discarded after three days, or use time in lieu of temperature (TILT), which limits permitted serving time to one day. Rather than adhere to a particular temperature guideline, all salads must be discarded after a defined time threshold, which is typically one lunch period, or two to three hours. The three-day rule is implemented in order to use leftovers as quickly as possible in order to decrease the risk of foodborne illness, particularly targeting *Listeria monocytogenes*, a pathogen that can grow at refrigerated temperatures.

Because there is no current guideline in place to track temperatures of salads during service, the North Carolina Department of Public Instruction (NCDPI) requested help in collecting data to monitor these temperatures. NCDPI's first choice for guidance documents is recent research; however, missing from much of the literature are consistent, reliable data on actual conditions and practices of how leafy greens are stored and handled in food service kitchens. There are no data to suggest whether the 3-day rule is a good policy, or if there should be another standard in place to manage risk. Collecting real data is the most effective way to reduce assumptions. This research gathered temperatures of leafy greens in food service kitchens over time. This included storage in coolers, during meal preparation, and most notably, during service. Establishing temperature patterns of leafy greens during storage and service is imperative to understanding proliferation of pathogens. In order to ensure that an effective and standardized policy is in place, systems should not only remain below 5°C, but also show consistency in temperature between schools. A consistent policy that is

considered standardized across North Carolina will help to greatly reduce foodborne illness risk associated with serving leafy greens.

3.2 Materials and Methods

To collect data on temperatures of leafy greens in school kitchens, schools were recruited with the help of the School Meals Initiative (SMI) team. The SMI team recruited schools that serve cut leafy greens as part of their meal program to participate in the study.

A total of twenty-four elementary, middle, and high schools were visited in eleven counties across North Carolina through convenience sampling (Figure 3.1). The schools that participated were the first to agree to participate and to schedule a week to conduct the study. Table 3.1 lists a description of the schools that participated, based on school level, school attendance, and location (rural or urban).

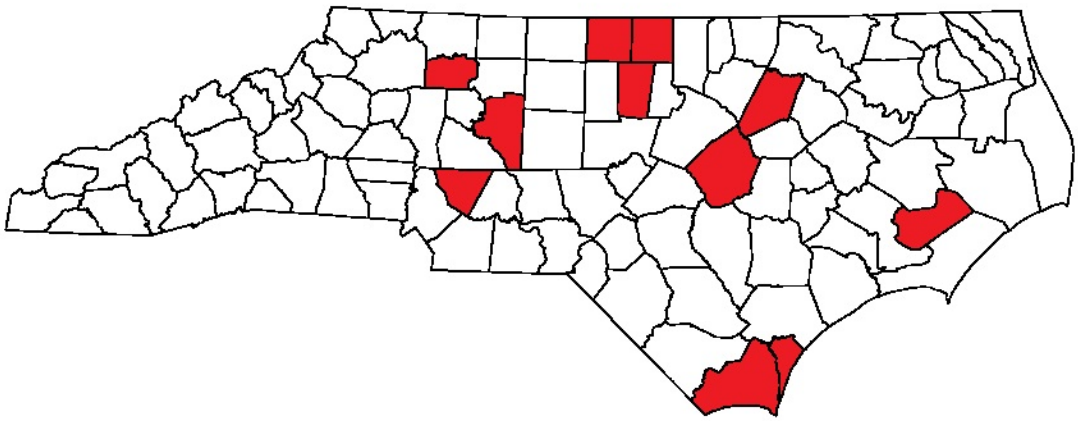

Figure 3.1. Location of schools participating in study. A total of twenty-four schools participated in eleven counties across the state.

The number of salads prepared each day varied by school. Depending on the school and type of serving line, the salads were either put back in the storage cooler or into a prep cooler close to the serving line for easy accessibility when lunch began. Figure 3.2 illustrates

the pathway of leafy greens in a school kitchen. The main focus in this study was the temperature fluctuation in the time from holding cooler to serving line and back, as DPI believed temperatures were reaching above the recommended 5°C.

Table 3.1. School information of participants- school level, attendance numbers, and location.

Level	Attendance	Rural/Urban
High	1400	Rural
Middle	650	Rural
Middle	530	Rural
High	1,100	Rural
High	660	Rural
High	1750	Urban
High	1100	Urban
High	500	Rural
Elementary	500	Urban
Middle	600	Urban
Middle	750	Urban
Middle	800	Urban
Elementary	625	Urban
High	850	Rural
Middle	750	Rural
Elementary	675	Rural
Elementary	630	Rural
High	1150	Rural
High	1500	Rural
High	1,050	Rural
Middle	900	Rural
Elementary	1,175	Rural
Elementary	990	Rural
High	970	Rural

Two main policies of NCDPI's School HACCP program define storage and serving of leafy greens: time in lieu of temperature (TILT) and the 3-day rule. The TILT system is based on the FDA's designation of foods as potentially hazardous that require time/temperature control for safety (TCS) to prevent pathogen growth (FDA, 2009). TILT implements an upper time threshold for which salads can be out on the serving line, and they must be thrown away after that time point. Typically, the time threshold is one full lunch

serving period. Of the twenty-four schools in this study, six used the TILT system. Figure 3 illustrates the TILT system. The remaining eighteen schools follow a rule that specifies that a salad may be put out for sale for a total of three days, and must be discarded at the end of the third day. For example, if a salad is prepared on a Monday, it may be put out to be sold on Monday, Tuesday, and Wednesday, but must be discarded at the end of the lunch period on Wednesday. This is known within the NCDPI system as the 3-day rule. The 3-day rule follows the path seen in Figure 3.2.

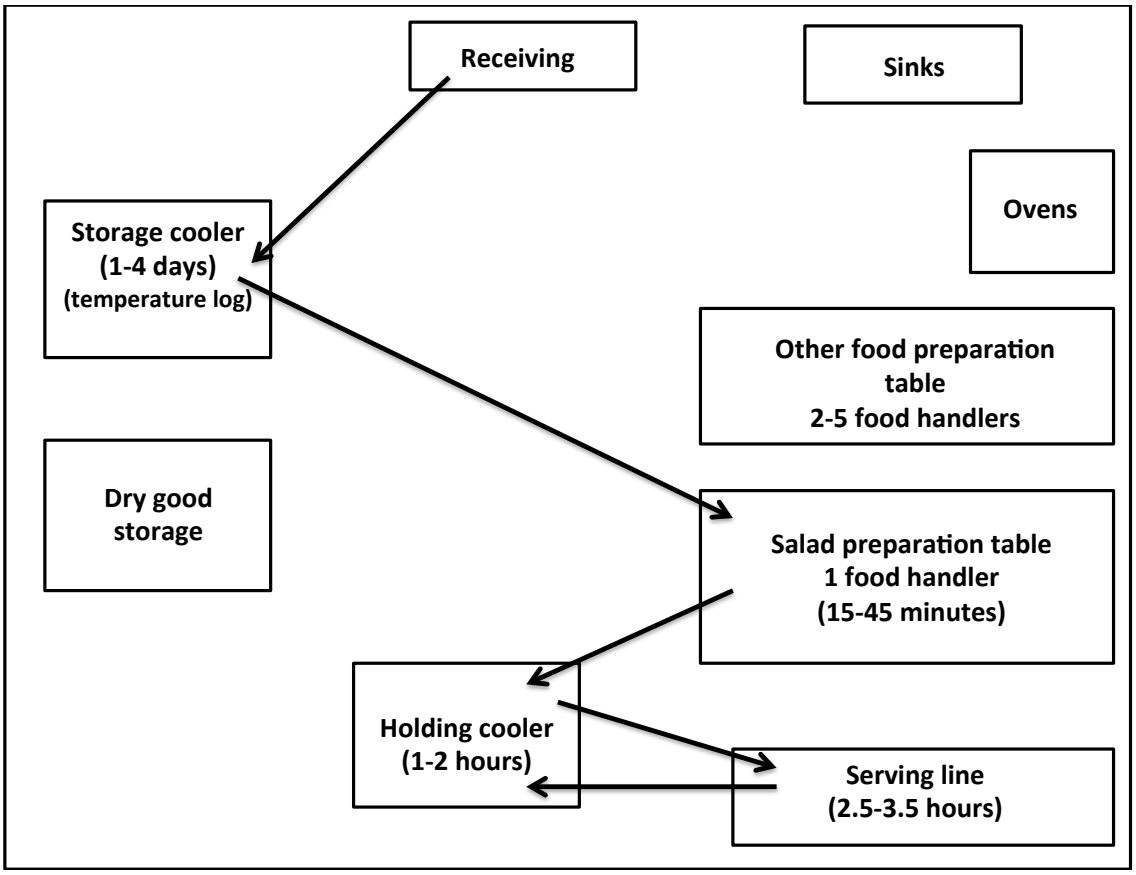

Figure 3.2. Generic schematic of preparation and storage of leafy salads in a school kitchen prior to sale. Approximate time at each station is noted.

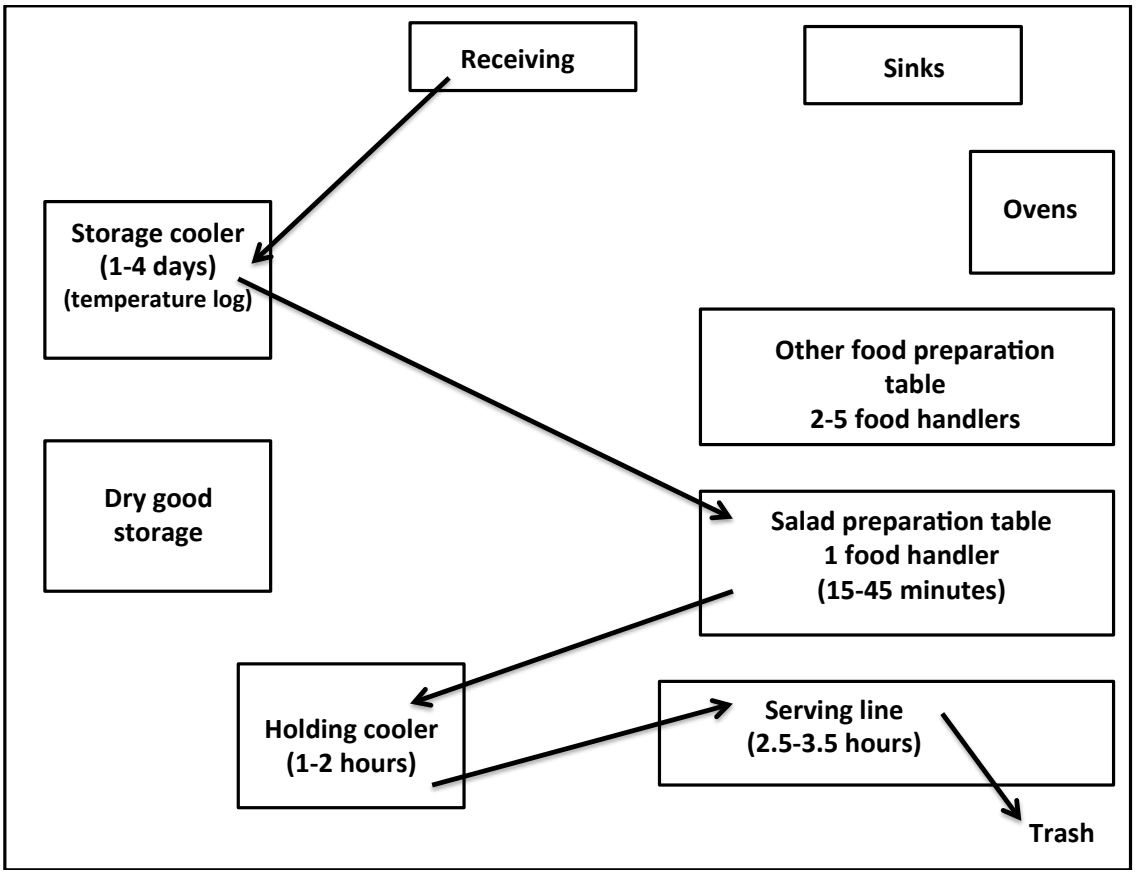

Figure 3.3. Generic schematic of preparation and storage of leafy salads in a school kitchen prior to sale in the TILT system. Approximate time at each station is noted.

A school may be classified as TILT after filling out the TILT classification application, which includes descriptions of the ingredients, procedure for making the food, and designation of the time controls involved in holding. The environmental health department reviews the application and works with the school to set up the proper process and define the upper time threshold for the particular food. Once this application is approved, that particular food is designated as TILT for that school.

Recording temperatures

Temperatures of leafy greens were recorded on the serving line over the entire lunch period. LASCAR EL-USB-1 data loggers were used to track temperatures in this study. This data logger unit was selected based on its size, ability to store a large amount of data, and ease of downloading data. A test salad was prepared by placing leafy greens in a clamshell container; the same amount was used as in the standard size of salads for sale. A probe attached to the data logger was inserted into leaves in the center of the container, and the data logger on top inside the container (Figure 3.4). The clamshell container was labeled "test" to prevent accidental sale of the product and loss of data.

Figure 3.4. Picture of data logger set up in clamshell container. Probe was inserted into leaves in center of container.

The kitchen staff was told that the purpose of this project was to monitor the temperature of the salads being served; it was emphasized that this information would not be sent to the health departments or reported in any way outside of research purposes. Kitchen managers were instructed to keep test salads with the rest of the salads served that day in

order to achieve the most accurate data possible. Test salads were kept in the holding cooler, put out on the serving line, and placed back in the holding cooler, if applicable. The majority of the lunch periods lasted approximately 2 to 2.5 hours. As a result, data loggers were programmed to record the temperature of the clamshell every 5 minutes for approximately 3.5 hours, or 210 minutes, for 3 days. The 3.5-hour data-recording period allowed for the establishment of a baseline temperature on either side of the serving period to see the full amount that the temperature increased.

Pathogen Modeling Program

The USDA Agricultural Research Service Pathogen Modeling Program (PMP) Online was used to predict aerobic growth of *E. coli* O157:H7 in salads based on the temperatures collected of each serving system. Different temperatures were entered in order to model the temperatures of the different serving systems recorded in the schools (Table 3.2). Default values of the program were used for the other parameters, which included pH (6.5), sodium chloride percentage (0.5%), sodium nitrate content (0 ppm), and initial level of bacteria present (3.0 LOG cfu/ml). Growth data for each temperature was generated by the program, which included lag phase duration, generation time, growth rate, and maximum population density.

Table 3.2. Temperature profiles generated for PMP with corresponding cooling system.

Temperature Profile	Cooling System
6.5°C	Cooling well Healthy Cart
7°C	Cooling plate Cooling well Display cooler
8.5°C	Cooling well Ice
10°C	Cooling well
10.5°C	Loose, cooling pan

3.3 Results

There were seven different types of cooling systems used in school serving lines of the 24 schools visited (Table 3.3). Cooling wells were the most common system in our study (10 total, 2 TILT). Five schools (2 TILT) used display coolers having juice, milk, and other refrigerated items. Four schools used portable carts on wheels, called "Healthy Carts," in which product is kept in an insulated box underneath the display portion, and a few salads at a time are placed on top in a small cooling well. One school kept salads in an ice bath and replaced ice when the ice began to melt; this was a TILT system. One school used a cooling plate, which is a cold electric surface built into the service counter and salads are placed on top of it. One school did not place salads out on the serving line at all, but stored them in a holding cooler and students asked a cafeteria worker when they wished to purchase a salad. There were two cases where clamshell containers were not used at schools. In these cases, leafy greens were served loose in cooling pans within a cooling well, and students served themselves or a person worked the line specifically for salads. One of the two was a TILT system.

Table 3.3. Cooling systems of participating schools with total number and total TILT schools.

Cooling system	Number of schools	TILT
Cooling plate	1	0
Cooling pan, loose	2	1
Ice	1	1
Cooling well	10	2
Display cooler	5	2
Healthy cart	4	0
Holding cooler	1	0
Total	24	6

After collecting temperature data points over the 3-day period, data was analyzed to determine the frequency of total time that the salads spent above 5°C. Table 3.4 lists the frequencies of total temperatures above 5°C by cooling system.

Table 3.4. Total frequency above 5°C by cooling system.

Cooling system	Number of schools	Total recorded time	Average time above 5°C	Standard deviation	Average frequency above 5°C
Cooling plate	1	555 minutes	394 minutes		71%
Cooling pan, loose	1	735 minutes	477 minutes		63%
Cooling well	8	640 minutes	224 minutes	208 minutes	35%
Display cooler	3	640 minutes	217 minutes	101 minutes	34%
Healthy cart	4	650 minutes	52 minutes	52 minutes	8%
Holding cooler	1	645 minutes	0 minutes		0%

Cooling wells were the most commonly used cooling method of the schools that participated (n=10). They showed temperatures above 5°C 35 percent of the time; two of these were TILT schools (Figures 3.5). Gaps in the data occur when measurements were not accurately recorded, or if changes during the lunch periods affected the recording time. Four schools used Healthy Carts, showing temperatures in excess of 5°C 8 percent of the time (Figure 3.6). Display coolers were used by 4 schools and found to be in excess 34 percent of the time; two of these were TILT schools (Figure 3.7). The cooling plate method (n = 1) showed temperatures in excess 71 percent of the serving time (Figure 3.8). One school used a holding cooler, and did not show any temperatures in excess of 5°C during the serving time (Figure 3.9). It was the only school that did not show any temperatures above the acceptable cut-off temperature. One school used ice, which showed temperatures above 5°C 49 percent of the time; it was also a TILT school (Figure 3.10). Two schools used a cooling pan in

which leafy greens were served loose (not in clamshell containers), and showed temperatures above 5°C 63 percent of the serving time; one of those schools was TILT (Figure 3.11).

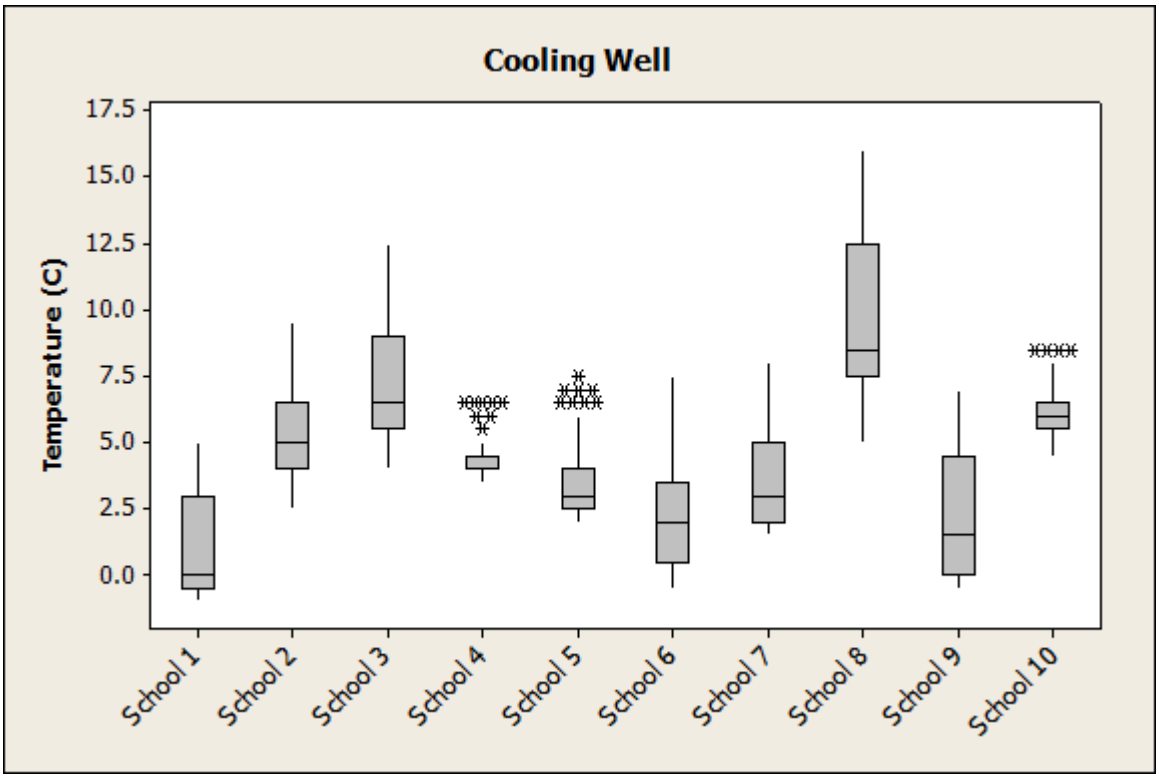

Figure 3.5. Temperatures of leafy greens held in cooling wells during serving time over 3 days (n = 10). Two schools were TILT. Standard deviation = 3.03°C. Asterisks denote extreme outliers.

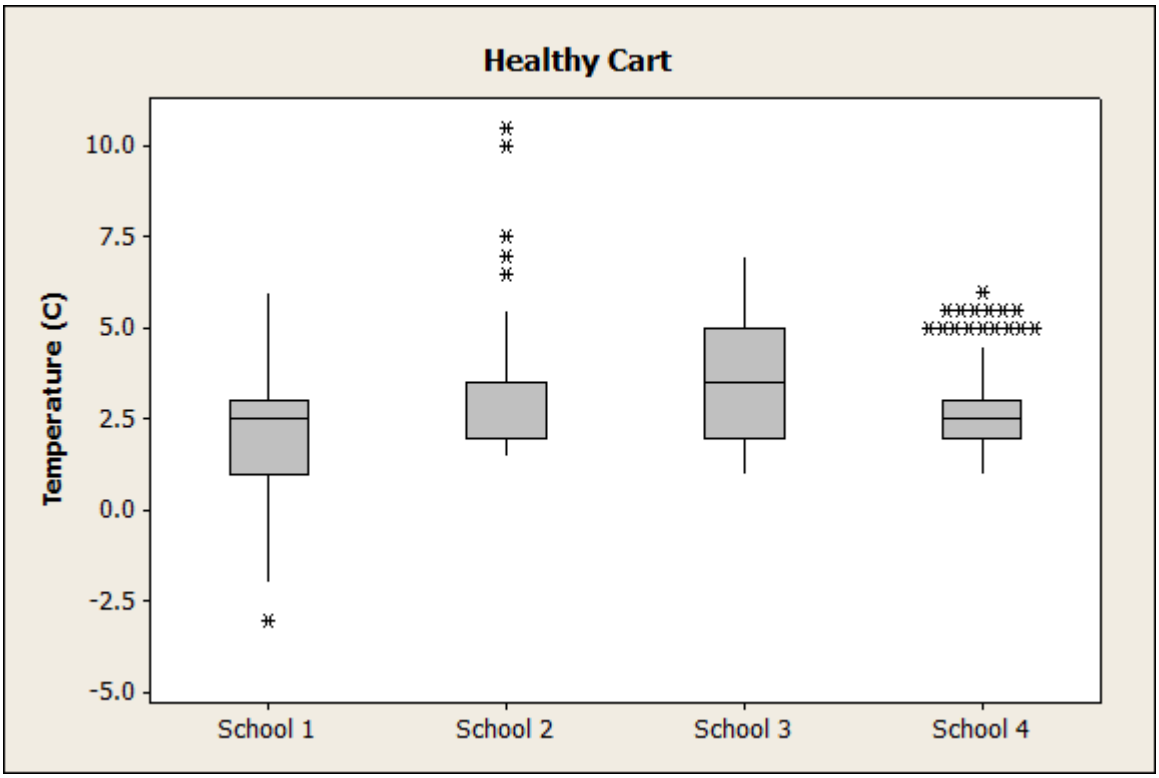

Figure 3.6. Temperatures of leafy greens held in Healthy Carts during serving time over 3 days (n = 4). Standard deviation = 1.59°C. Asterisks denote extreme outliers.

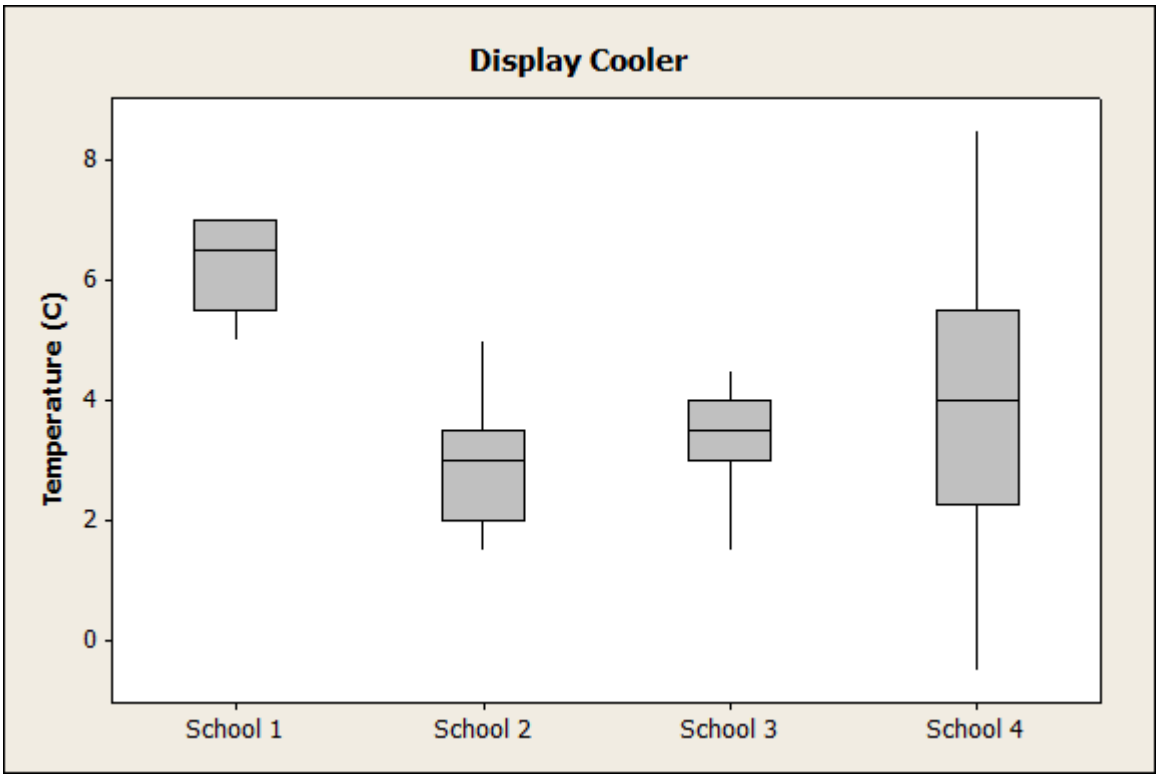

Figure 3.7. Temperatures of leafy greens held in display coolers during serving time over 3 days (n = 4). Two schools were TILT. Standard deviation = 1.60°C.

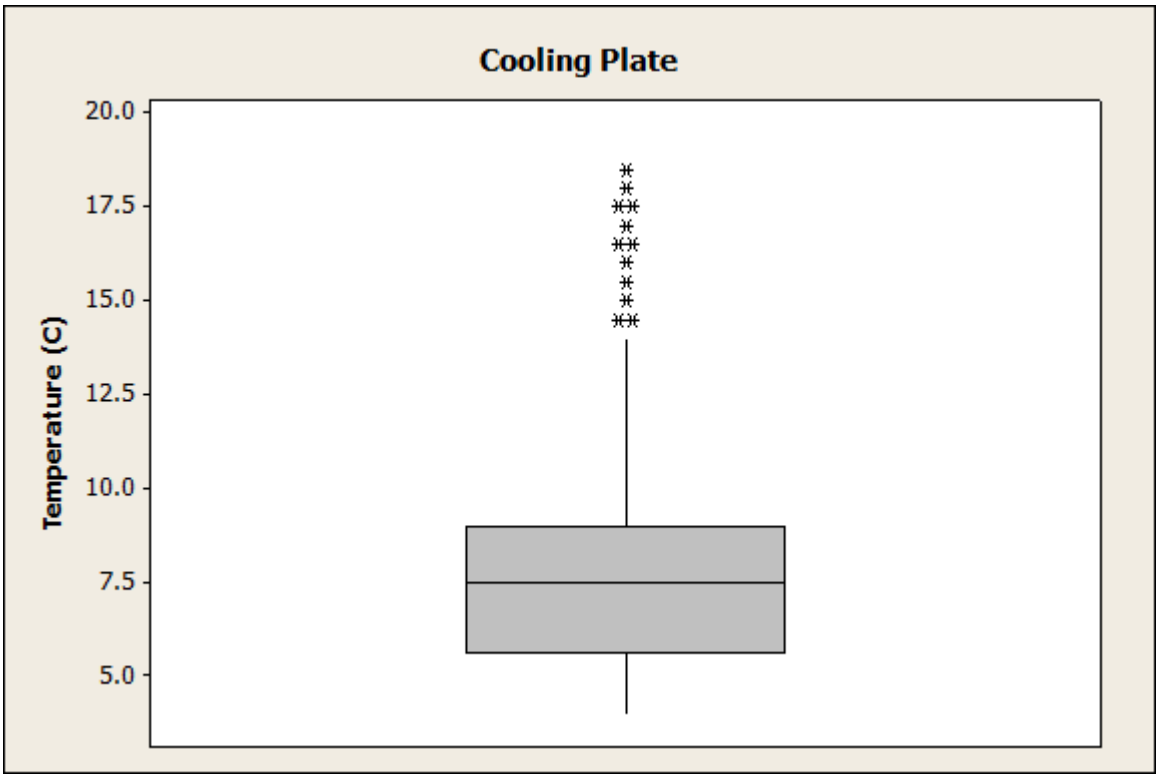

Figure 3.8. Temperatures of leafy greens held on cooling plate during serving time over 3 days (n = 1). Asterisks denote extreme outliers.

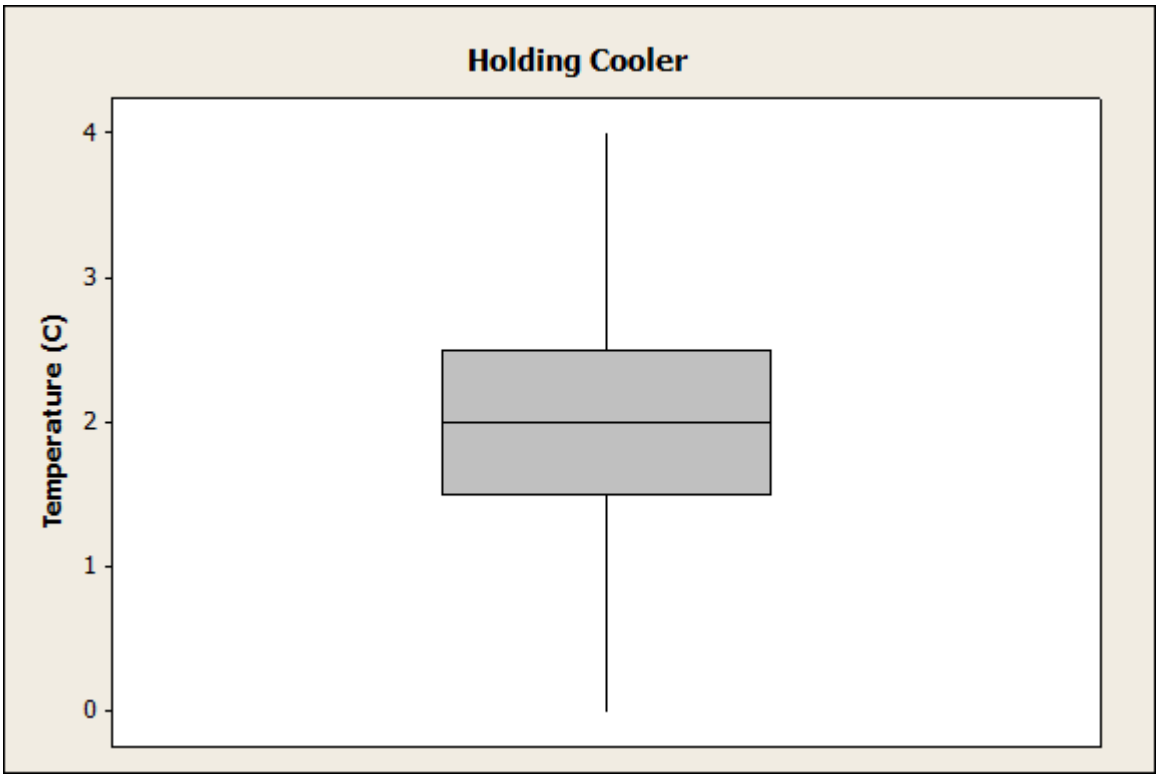

Figure 3.9. Temperatures of leafy greens held in holding cooler during serving time over 3 days (n = 1).

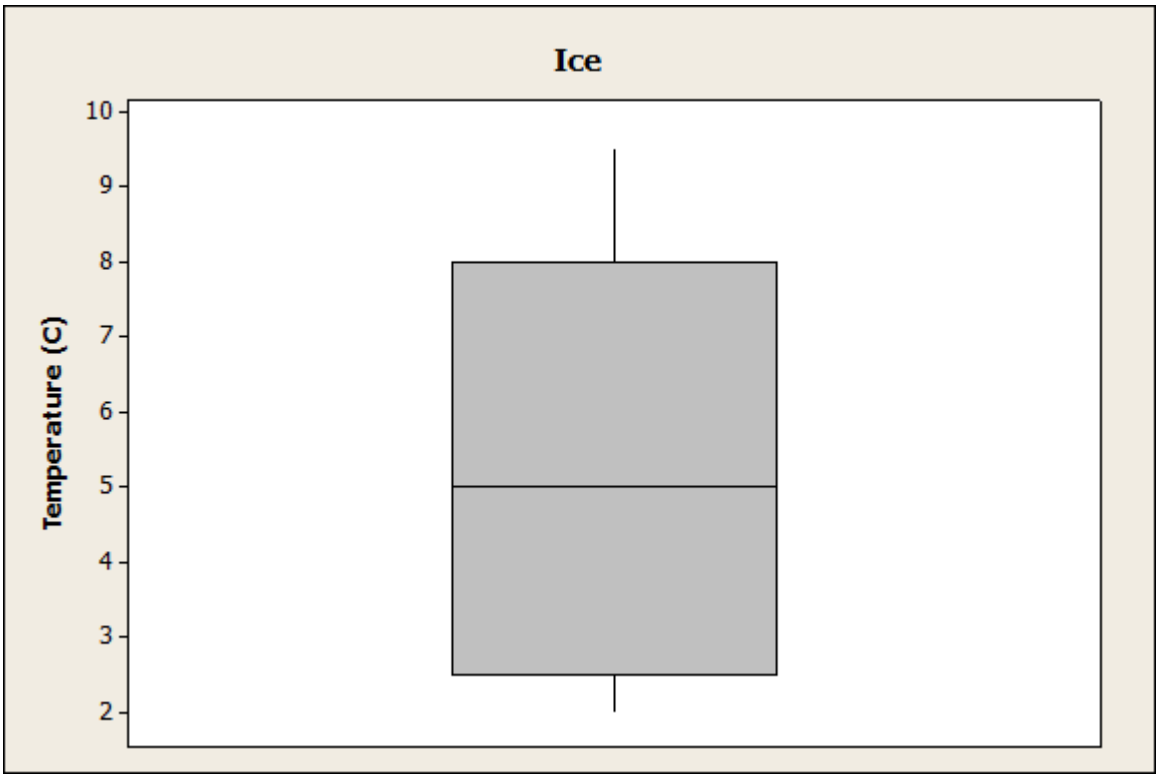

Figure 3.10. Temperatures of leafy greens held on ice during serving time over 3 days (n = 1). This school was TILT.

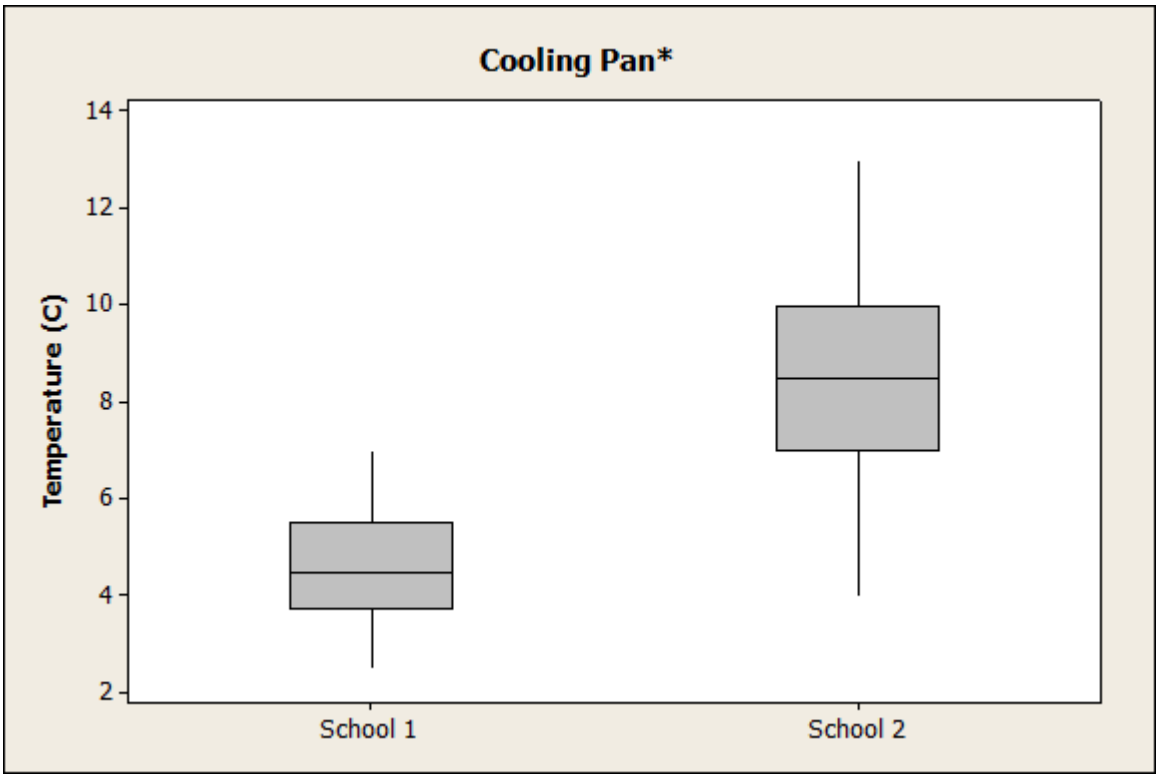

Figure 3.11. Temperatures of leafy greens held in cooling pans during serving time over 3 days (n = 2). The asterisk (*) denotes that leafy greens were served loose in the pan, not in a clamshell container. One school was TILT.. Standard deviation = 2.49°C.

Temperature profiles were generated based on the data collected and input into the Pathogen Modeling Program (PMP). In the case that a pathogen is present, no cases generated by the model resulted in a lag phase and generation time that were short enough to allow for growth during serving time (Table 3.5).

Table 3.5. Growth data output from Pathogen Modeling Program based on temperature profiles of serving systems.

Temperature profile	Lag Phase Duration (hours)	Generation Time (hours)	Growth Rate (log (cfu/ml)/h)	Maximum Population Density (log (cfu/ml)
6.5°C	138.52	10.74	0.028	9.40
7°C	120.28	9.67	0.031	9.40
8.5°C	79.78	7.10	0.042	9.40
9.5°C	61.35	5.83	0.052	9.40
10°C	61.35	5.83	0.052	9.40
10.5°C	47.58	4.81	0.063	9.40

3.4 Discussion

Maintaining cut leafy greens at or below 5°C is an important factor in controlling growth of pathogens if they are present on a product. Storing cut leafy greens above 5°C has been shown to allow for the growth of pathogens such as *E. coli* O157:H7 (Abdul-Raouf et al., 1993; FDA, 2009; Khalil and Frank, 2010; Li et al., 2001; Lopez-Velasco et al., 2010; Luo et al., 2009).

The objective of this study was to determine whether there was a difference in temperatures of leafy greens between the 3-day rule and the TILT system. Results from this project show that the majority of salads held in clamshell containers served in the participating school cafeterias reach temperatures above 5°C, the acceptable cut-off point set by the U.S. Food and Drug Administration Model Food Code. The amount of time salads

spend above the acceptable temperature plays a crucial role in how much pathogens could proliferate. For example, 2 hours spent above 5°C will allow for more bacterial growth than 20 minutes above 5°C.

The temperatures of the leafy greens followed a distinct pattern for all of the cooling methods; there was an increase in temperature during serving time for all systems. However, there was a wide degree of variability in how much the temperature increased and the rate of increase both within and between systems. For example, the cooling wells and Healthy Carts showed a smaller range of temperature increase during the lunch period, in comparison to schools that used ice and cooling pans, which showed the largest increase in temperature for each day.

Systems that keep salads in a cooling well and the Healthy Cart also showed the lowest total frequencies above 5°C during serving. Salads served in cooling wells reached above 5°C approximately 35 percent of the time, and those stored on ice reached above 5°C nearly half of the time. Salads served on a cooling plate system showed temperatures in excess of 5°C for the highest percentage of time.

Although there are trends in the data, there is not enough data collected for each cooling method to definitively state which cooling methods are better at keeping salads below 5°C than other methods; the data collected so far is just to begin showing what temperatures are occurring. Another limitation of the study is that it is difficult to predict how quickly salads may sell in a given day; this can vary based on how many lunches are being sold and other food being sold that day. As a result, if a low number of salads are sold on a given day, there will be salads remaining to be served the next day, increasing the time at which they are exposed to the increase in temperature.

Temperature abuse during storage has played a role in contributing to foodborne outbreaks (CDC, 2011). This study demonstrates that temperatures reach above 5°C during serving in many cases. Under the 3-day rule, salads could be exposed to temperatures above 5°C for 3 subsequent days and a total of several hours overall. However, even though

temperatures may be out of compliance, based on data from the PMP, the temperatures seen in these serving systems do not appear to pose a significant risk.

If schools are concerned about adhering to temperature regulations, TILT could be used as the primary model to update the 3-day rule. The TILT system sets a maximum time that a product may be served within one serving period, removing the re-serving option for leafy greens. TILT decreases the total amount of time that cut leafy greens could spend above 5°C. Collecting this temperature information provides the evidence that temperatures of leafy greens do reach above 5°C during serving in most of the serving systems.

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CHAPTER 4
TRACING TEMPERATURE PATTERNS OF CUT LEAFY GREENS DURING TRANSPORTATION

4.1 Introduction

Until 2012, North Carolina commercial food service businesses were regulated by a set of standards established in 1976 and updated periodically as new scientific information was revealed. As of September 1, 2012, North Carolina will adopt the U.S. Food and Drug Administration's Model Food Code (Food Code) by reference, which means that the 2009 Food Code will be adopted with amendments, additions, and deletions (Charlotte Observer, 2012). The Food Code is a guidance document for retail establishments based on scientific evidence that addresses risk factors that cause foodborne illness. The Conference for Food Protection (a panel of academics, industry representatives, and other scientists) works to keep the Food Code up-to-date and amends it every two years. North Carolina is the last state to adopt a 1993 or later edition of the Food Code (AFDO, 2012).

The Food Code includes a requirement that fresh-cut leafy greens, items such as lettuce and spinach that have been cut beyond the root zone or the harvest cut, be recognized as a Potentially Hazardous Food (PHF) and must be received at restaurants at or below 5°C (FDA, 2009). This ensures that the product has been temperature controlled after harvest, through processing, during transportation and to delivery in case contamination has occurred. During the process to adopt the food code, public town hall meetings were held to explain what changes to the Food Code would mean for the industry and to receive feedback on the revisions. These meetings revealed a large amount of resistance to the new Food Code in rural communities (CFSA, 2012). Farmers who grow cut leafy greens were concerned because of the potential for further cost in cooling equipment for transportation (CFSA, 2012). Some members of the public questioned who had the authority to enforce the new changes to the Food Code, which is a complicated question. The North Carolina Department

of Agriculture & Consumer Sciences (NCDA&CS) regulates processing under 21 CFR 110, and so processors follow under the jurisdiction of the NCDA&CS. The North Carolina Department of Health and Human Services regulates the retail food service industry. According to this system of regulation, technically, the supplier does not fall under the jurisdiction of the Food Code. However, this new regulation directly impacts both the supplier and the food service establishment.

In addition to the cost and legal issues raised, the public was skeptical about the validity of the science behind the ruling, suggesting that this new regulation would be an unnecessary burden and may not make food any safer (The Packer, 2012). Farmers and restaurateurs stated that this regulation is more applicable to large companies that transport leafy greens for long distances of time, not small farmers in North Carolina, who may be transporting their product for a maximum of four hours, but typically even less time than that (The Packer, 2012).

Temperature control reduces the likelihood of pathogen growth, in this case *E. coli* O157. Cutting the leaf exposes plant tissue, provides nutrients for bacteria and allows for pathogen growth when kept at temperatures above 5°C (FDA, 2010). There have been numerous outbreaks associated with cut leafy greens; 53 have been reported since 1995 (CDC, 2011). *E. coli* O157:H7 is the primary pathogen of concern, due to its ability to attach to cut portions of the leaf (Takeuchi and Frank, 2000). *E. coli* O157:H7 has also proved capable of growing at temperatures above 5°C (Khalil and Frank, 2010; Lee and Baek, 2008; Luo et al., 2009). Danyluk and Schaffner (2011) modeled this data, showing that cut leafy greens could undergo a one-log growth each day at optimum temperature, approximately 37°C.

Leafy greens are available in North Carolina throughout most of the growing season (typically April through November). Once leafy greens reach maturity, they are harvested by hand and cut. They are then brought to processing, where the product is immersed or sprayed with cold water, known as hydrocooling. They are stored at refrigerated temperatures until

they are transported. The storage time varies with farmers, depending on how long the time gap is between harvesting and transportation. If contamination occurs at any point prior to transport, any further temperature abuse of the product could result in an even greater problem. Time plays a large role in this issue; a longer time above 5°C will allow for more time for pathogens to proliferate because conditions are closer to what bacteria prefer for growth. For example, 3 hours above 5°C will allow for a greater increase in bacteria growth than 20 minutes above 5°C.

This study was conducted to determine whether farmers' concerns were valid and actually based on data. Using coolers with ice or ice packs purchased at a local grocery store is a much cheaper alternative to buying more elaborate cooling equipment or a refrigerated truck space. If these methods were capable of keeping product below 5°C, this information could be relayed to small farmers as an option for transporting their product. The best way to answer this question was to record temperatures of cut leafy greens over a period of several hours in order to monitor the temperature patterns of the leafy greens.

4.2 Materials and Methods

In order to evaluate the impact of the Food Code on leafy greens suppliers, a project was designed to determine whether a relatively inexpensive system, commonly available coolers from food retail outlets, are capable of keeping leafy greens below 5°C during transportation. To explore this, cut leafy greens were held in coolers with ice and ice packs for four hour time periods to mimic conditions that would be expected during delivery from a farm to a restaurant. Leafy greens were purchased from farms in Pittsboro and Carthage, NC for a market cost of $60 for 6.8 kilograms. Leafy greens were sorted by the producer and stored in plastic storage bags of approximately 0.34 kilograms.

Three coolers were compared for their effectiveness at holding leafy greens below 5°C: a 15-liter Igloo, a 28-liter Styrofoam and a 66-liter Coleman. The coolers were purchased at Wal-Mart; the small Igloo cost approximately $15, the Styrofoam cooler cost

approximately $5, and the large Coleman cost approximately $40. Two different cooling methods within each cooler were also compared for their effectiveness: ice and ice packs. Ice packs used were Coleman brand, purchased at Wal-Mart and 14 centimeters by 19 centimeters. Table 4.1 lists the cost associated with each holding method.

Table 4.1. Average cost for each system to transport leafy greens. Ice packs are one time costs, while ice is either a recurring cost or added labor to make the ice. Approximately 3 kilograms were held in the Styrofoam cooler, 3.85 kilograms in the small Igloo, and 8.5 kilograms in the large Coleman.

Cooler	Cost of cooler	Cost of ice/ice packs	Total cost
Styrofoam	$4	$2/$6	$4-10
Small Igloo	$18	$2/$6	$20-25
Large Coleman	$40	$5/$14	$45-55

Each cooler was filled with as many leafy greens that could fit inside the cooler without crushing the product. Approximately 8.5 kilograms of leafy greens were held in the large Coleman, 3 kilograms in the Styrofoam, and 3 kilograms in the small Igloo. Six ice packs were used in the Styrofoam (Styrofoam/ice packs) and small Igloo (small/ice packs), and fourteen ice packs were used in the large Coleman (large/ice packs). This number of ice packs could cover the bottom of the cooler, and allow for three ice packs between each layer of leafy greens. Approximately 2 kilograms of ice were used for the Styrofoam (Styrofoam/ice) and small Igloo (small/ice), and 6.8 kilograms of ice were used for the large Coleman (large/ice). The amount of ice was based on a layer of ice on the bottom of the cooler, and a layer of ice between each layer of leafy greens. Table 4.2 lists the amount of ice and ice packs used for each holding system.

For each trial, approximately one layer of ice or one layer of ice packs was added to the bottom of each cooler. One layer of bagged leafy greens was then placed in the cooler. Another 0.5 kilogram of ice or one layer of ice packs were put over the bagged leafy greens, then a second layer of bagged leafy greens were put in the cooler. For the small and Styrofoam coolers, there were two layers of leafy greens. Each layer had three bags. The

large cooler had three layers of leafy greens. Each layer had eight bags. In each case, another layer of ice or ice packs were placed over the top layer. Figure 4.1 illustrates the set-up of the coolers with ice. Figure 4.2 illustrates the set-up of the coolers with ice packs. The cooling layer on top has potential to damage product; using this set-up in this study may be a limitation if a farmer wants to avoid that possible damage.

Table 4.2. Types of coolers used to hold leafy greens, with number of ice packs and amount of ice used for each trial.

Cooler	Ice packs	Ice (kilograms)
Large Coleman	14	8.5 kilograms
Small Igloo	6	3 kilograms
Styrofoam	6	3 kilograms

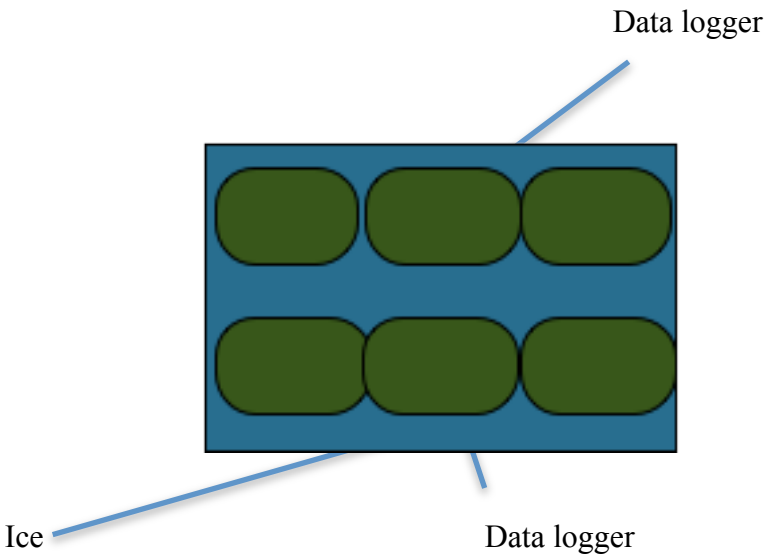

Figure 4.1. Arrangement of coolers with ice. Green circles signify bagged cut leafy greens; blue signifies ice.

Before running each trial, the temperature of leafy greens were checked to ensure it was less than or equal to 5°C. Table 4.3 lists the starting temperatures for each trial.

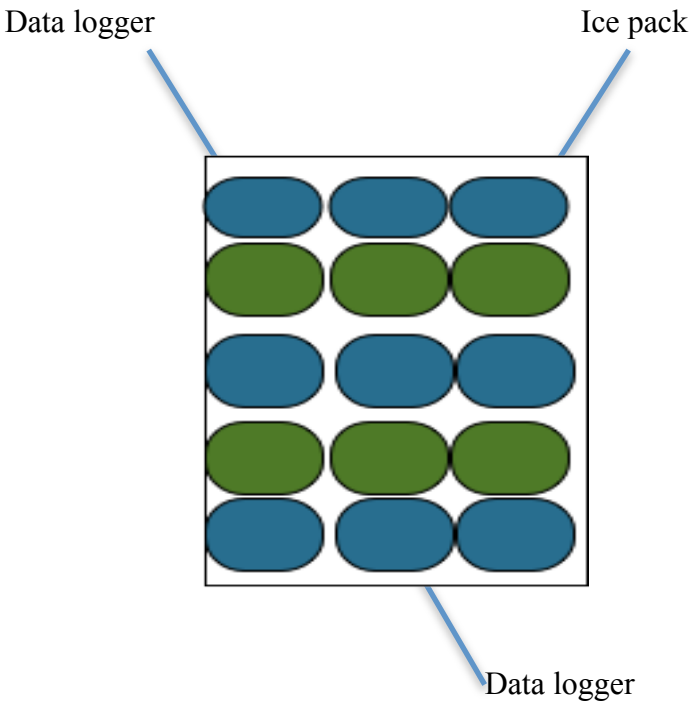

Figure 4.2. Side view of arrangement of coolers with ice packs. Green circles signify cut leafy greens; blue circles signify ice packs.

Table 4.3. Starting temperatures for each holding method. Average starting temperature = 3.36°C. Standard deviation = 1.09°C.

Trial	Average Starting Temperature (°C)
Large/ice	4.14°C
Small/ice	1.53°C
Styrofoam/ice	2.5°C
Large/ice packs	3.83°C
Small/ice packs	3.98°C
Styrofoam/ice packs	4.16°C

LASCAR EL-USB-1 data loggers were used to track temperatures in this study. This data logger was selected based on its size, ability to store a large amount of data, and ease of downloading data. A probe attached to the data logger was inserted into leaves in one bag of leafy greens. Three data loggers were used in the largest cooler; one was placed in the bottom

layer of leafy greens, one in the middle layer, and one on the top layer. The data logger was placed in the center bag of each layer. Two data loggers were used in the smaller two coolers- one in the bottom layer and one in the top layer. The ambient temperature of the trunk was also recorded. The coolers were kept in a car trunk for four hours in order to mimic temperatures that could be expected during transportation. To mimic the most extreme conditions that could occur during transportation, the car was parked in the sun. Data loggers were programmed to record the temperature every 5 minutes for 4 hours in order to capture the temperature trend for the maximum amount of time a small-scale supplier could be transporting leafy greens. This was repeated a total of three times for each cooler.

4.3 Results

The average temperature for each cooler over the four hours, for each of the three trials, was determined. Figure 4.3 shows the average temperature of each cooler with ice over the four-hour period. All three coolers remained under 5°C for the four hours. Large/ice and Styrofoam/ice showed a decrease in temperature over the four hours, while small/ice showed an increase over the four hours. Given this data, a farmer could expect to be able to hold product under 5°C for at least four hours using ice. Figure 4.4 shows the average temperature of each cooler with ice packs over the four-hour period. There was an increase in temperature over the four hours for each cooler. Small/ice packs and Styrofoam/ice packs both reached above 5°C during the four hours. Figure 4.5 illustrates the maximum time length that a farmer could expect to hold product below 5C using ice packs at the given ambient temperature of 32C. Styrofoam/ice packs increased at the highest rate, reaching above 5°C after 40 minutes. Small/ice packs reached above 5°C after 90 minutes. Large/ice packs reached 5°C after approximately 220 minutes.

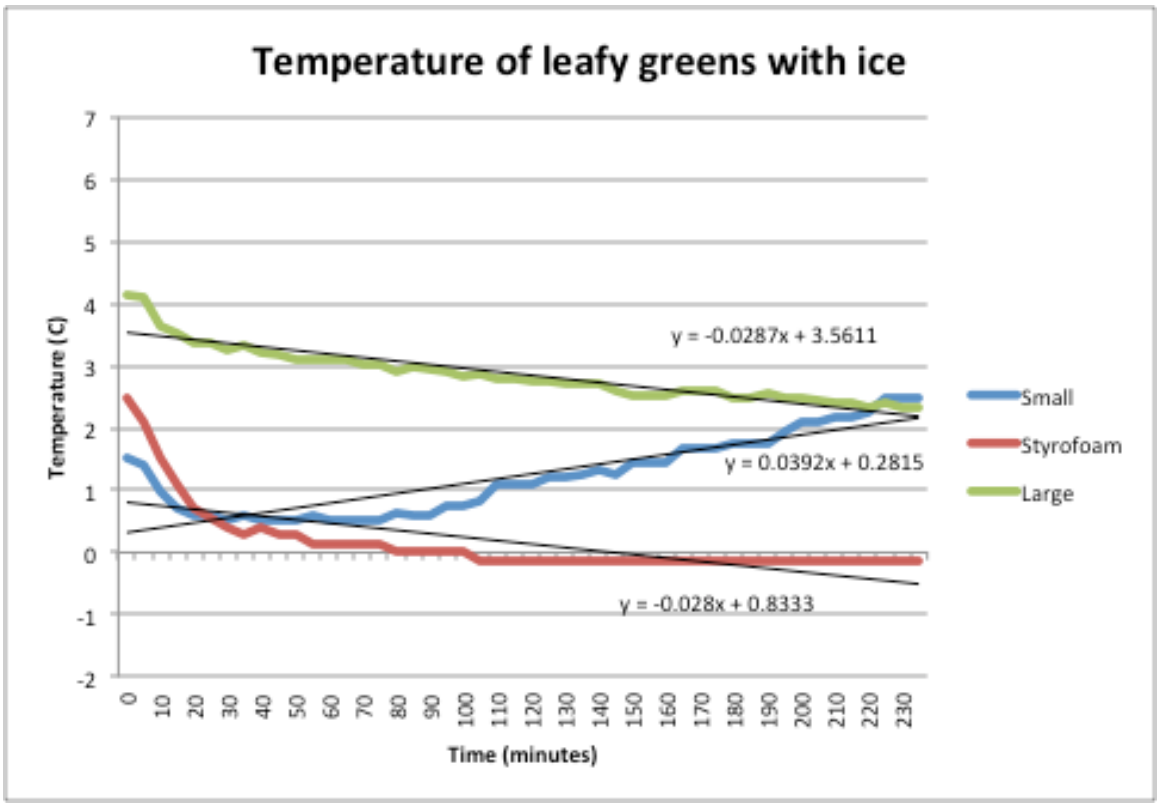

Figure 4.3. Temperature of cut leafy greens with ice. Leafy greens were stored in three different coolers over four hours. Average ambient temperature was 27°C.

The rate of temperature increase was calculated for each cooler. The equations for each holding system are listed in Table 4.4. Large/ice and Styrofoam/ice showed a decrease in temperature over the four hours. Small/ice and all coolers with ice packs showed an increase in temperature over time. Styrofoam/ice packs showed the largest increase over the four hours.

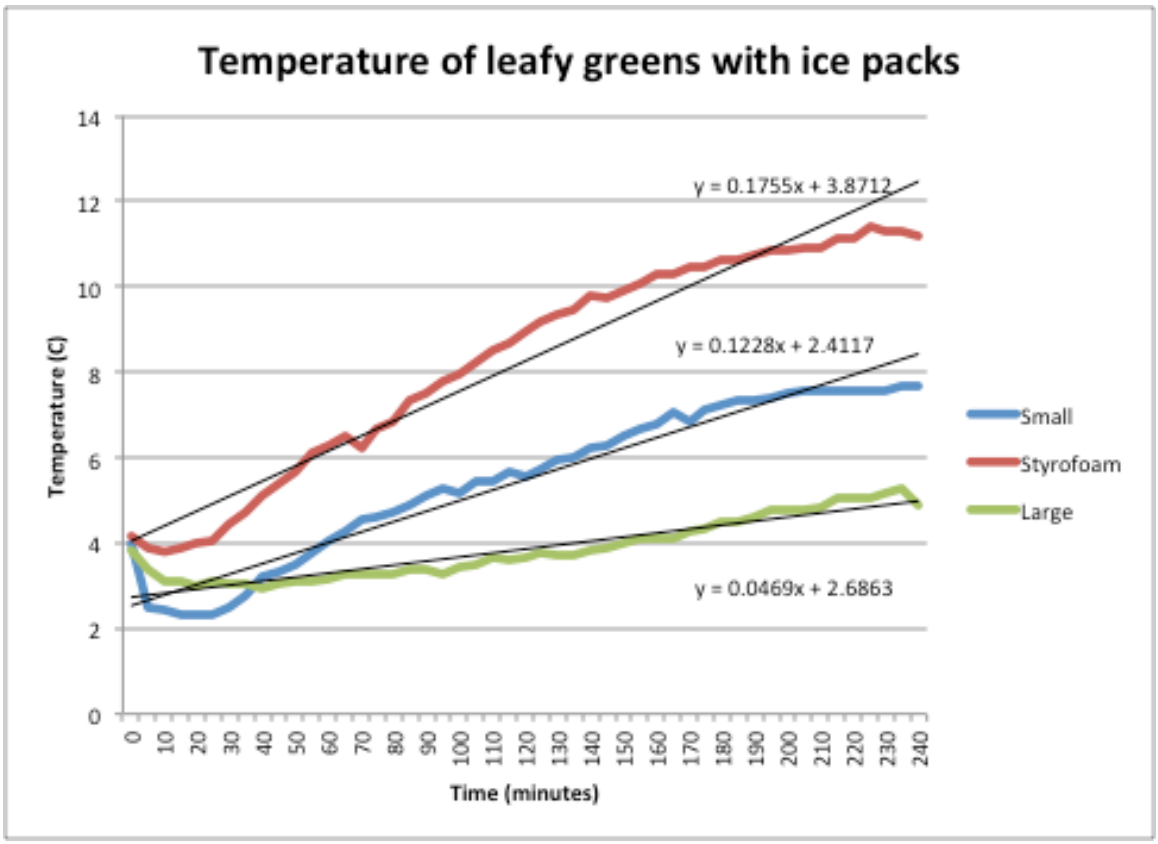

Figure 4.4. Temperature of cut leafy greens with ice packs. Leafy greens were stored in three different coolers over four hours. Average ambient temperature was 32°C.

An average ambient temperature was calculated for trials run for ice and ice packs (Table 4.5). The ambient temperature plays a crucial role in the rate of temperature increase; as ambient temperature increases, the rate of temperature increase inside the cooler increases, causing the product temperature to rise more quickly.

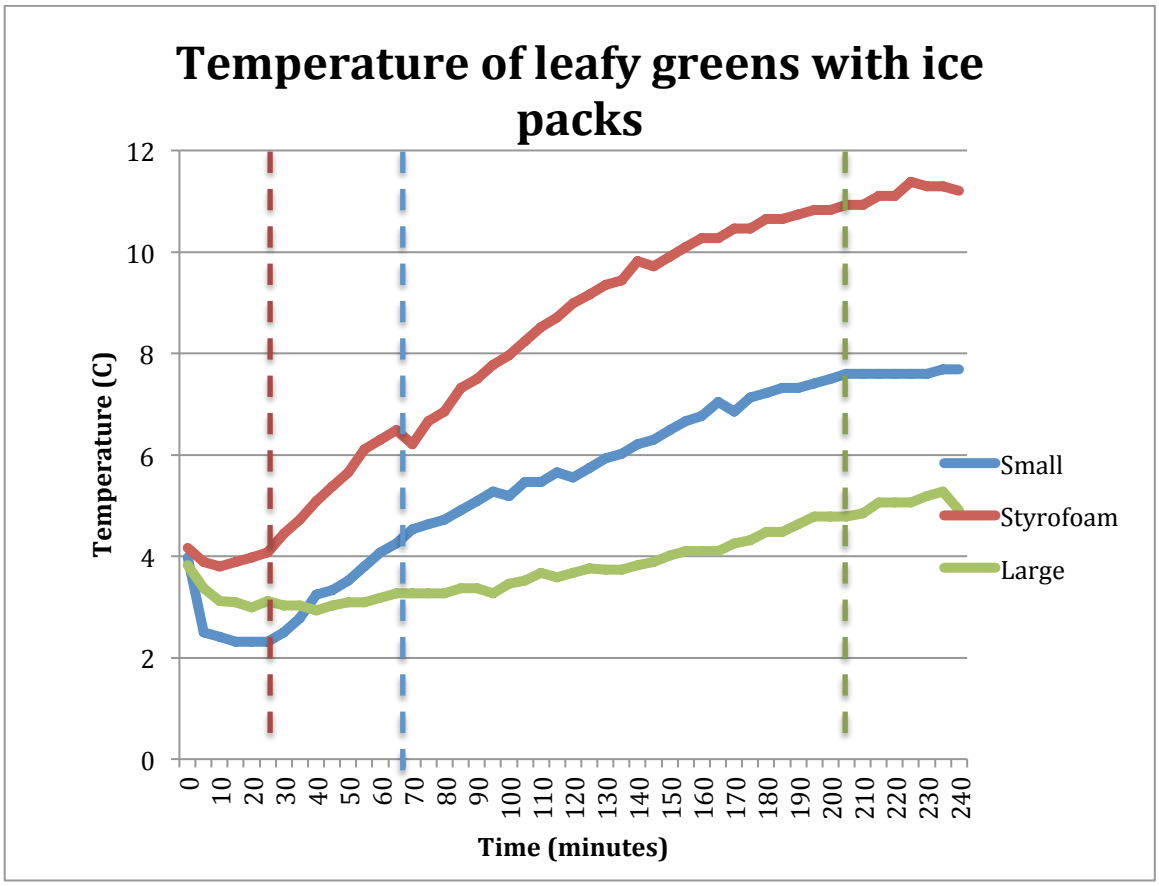

Figure 4.5. Temperature of cut leafy greens with ice packs. Dotted lines signify the point at which the temperature of the leafy greens exceeds 5°C, and match the corresponding color. Average ambient temperature = 32°C.

Table 4.4. Equations showing rate of increase for each trial.

Trial	Equation
Large ice	y = -0.0287x + 3.5611
Small ice	y = 0.0392x + 0.2815
Styrofoam ice	y = -0.028x + 0.8333
Large ice pack	y = 0.0432x + 2.5503
Small ice pack	y = 0.1228x + 2.4117
Styrofoam ice pack	y = 0.1755x + 3.8712

Table 4.5. Ambient temperatures by cold-holding method.

Cooling Type	Average Ambient Temp	Standard Deviation	Standard Error
Ice	27°C	5.58°	0.31°
Ice Pack	32°C	7.36°	0.35°

4.4 Discussion

Temperatures of cut leafy greens were monitored in coolers to determine if they were able to remain under 5°C.

Using ice as a temperature control method, all three coolers kept cut leafy greens at or below 5°C for the four-hour period. Large/ice and Styrofoam/ice produced a decrease in temperature over the four hours, while small/ice produced a slight increase in temperature. Assuming an ambient temperature of 27°C, leafy greens will remain below 5°C for up to four hours if stored with ice. Because ambient temperature has a direct impact on temperature inside the car trunk and the cooler, the temperature of cut leafy greens may increase more quickly at an ambient temperature higher than 27°C.

When using ice packs as a cooling method, only the large/ice packs kept temperatures below 5°C; both small/ice packs and Styrofoam/ice packs reached above 5°C during the four hours. Styrofoam/ice packs experienced the greatest average temperature increase over the four hours. Assuming an ambient temperature of 32°C, leafy greens will reach above 5°C within four hours if stored with ice packs. Figure 4.3 indicates the maximum time at which farmers could expect to hold cut leafy greens below 5°C.

There was a difference in cooling efficiency seen between the ice and ice packs. This may be due to the fact that ice is able to disperse evenly throughout the cooler and contact a higher product surface area. Ice packs have contact with a smaller amount of product. There could also be a difference in the coolers' ability to hold temperature based on their composition. The inside portion of Igloo coolers are composed of polypropylene and

insulated with polyurethane foam, which may be better at holding cold temperatures than Styrofoam. Using a cooler with ice is most efficient at keeping product below required temperature. The best volume to use would be dependent on the volume of product to be transported.

Coolers with ice were found to be effective methods for holding cut leafy greens below 5°C in a car trunk for four hours; in some trials, temperature even decreased over time. Price was a great concern for farmers resistant to the new rule; Table 4.1 lists estimated price by system. This method for holding leafy greens is applicable for anyone transporting or storing leafy greens for a long period of time: farmers' market vendors, produce stands, and consumers transporting their product. Although the new regulation within the Food Code caused controversy among North Carolina farmers, this method for cooling proved both effective in remaining below the temperature threshold and maintaining a relatively low cost.

There are some other safety issues to be considered when using ice as a holding method. Farmers may choose not to bag their product before putting it in the cooler; in this case, if ice were contaminated, it would make direct contact with the leafy green, resulting in cross-contamination. Contamination of ice could occur if a safe water source is not used or through equipment. Ice could also melt inside the cooler and pathogens could be introduced by another method. Even in the case of bagged product, contamination of the bag could occur, resulting in possible contamination of the product.

These findings show storing leafy greens in coolers with ice can keep product below 5°C, which is crucial for small farmers who transport fresh-cut leafy greens. There are several options for farmers when deciding which holding system to use in order to meet the new regulation within the Food Code (Table 4.1). Assuming delivery twice a week, a farmer could expect to spend approximately $520 per year in ice for maintaining a large cooler (in addition to the cost of the cooler), and approximately $210 per year for the small or Styrofoam coolers. Styrofoam coolers are less expensive than the other two coolers used in

this study; however, they are not as sturdy and a farmer would likely need to purchase several. As a result, maintaining a Styrofoam and small Igloo would be comparable in price.

Ice packs are not as effective at holding cut leafy greens below 5°C; it takes much less time to reach above 5°C. However, if a farmer is transporting product a short distance (40 to 90 minutes in the case of the Styrofoam and small cooler, respectively), ice packs could be used. This cost would be a one-time cost of approximately $25 total. Ice packs could be used in the large cooler for approximately 220 minutes; this cost would be approximately $55 for one time. Overall, the ice pack method is cheaper over time than ice, but not as efficient, particularly when holding cut leafy greens for longer periods. As discussed with the ice method, there is a chance of residual cross-contamination when using ice packs as well. This would be likely to occur when frequently re-using ice packs, particularly in coolers that hold products other than leafy greens.

One limitation of this study was that it compared only three coolers. In order to get a clearer idea of which cooler is best to use when transporting cut leafy greens, it would be useful to test several more types and sizes of coolers.

If leafy greens have been contaminated with a pathogen such as *E. coli* O157:H7, keeping their temperatures at or below 5°C should prevent further growth of the pathogen (Abdul-Raouf et al., 1993; Khalil and Frank, 2010; Lee and Baek, 2008; Luo et al., 2009). However, there are some situations where exceptions should be made to the receipt at 5°C rule. For example, some farmers harvest their leafy greens the same morning that they deliver. In many cases the time from harvesting to delivery is less than four hours. Because leafy greens are brought in from the field and may include a wash step, there may not be enough time to reduce the product temperature to 5°C before loading and delivery. As time and temperature are both factors for bacterial growth, the requirement that leafy greens be received at 5°C is more relevant to cases where there is a longer period of time between harvesting and delivery, and a longer time required for transportation.

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CHAPTER 5

CONCLUSIONS AND POLICY RECOMMENDATIONS

5.1 Summary

This study was conducted to investigate the effectiveness of a current North Carolina Department of Public Instruction's current temperature control and handling policies for leafy s are effective strategy to manage risk. Temperature abuse of leafy greens has played a contributing role in recent outbreaks (CDHS, 2007). As the process of cutting can release leafy green plant tissue, providing water and nutrients for bacteria to proliferate, U.S. Food and Drug Administration recommends that cut leafy greens be stored at 5°C (FDA, 2009; FDA, 2010). This recommendation is based on various studies that have demonstrated the ability of pathogens, particularly *E. coli* O57:H7, to grow at temperatures above 5°C in cut leafy greens (Abdul-Raouf et al., 1993; FDA, 2009; Khalil and Frank, 2010; Li et al., 2001; Lopez-Velasco et al., 2010; Luo et al., 2009).

Although a large amount of research has been conducted to determine factors contributing to contamination and growth from farm-to-fork, there is a gap in the research surrounding policies and practices of maintaining temperatures of leafy greens during transportation and preparation and service. Collecting data regarding storage temperatures of cut leafy greens is crucial to understanding the likelihood of pathogen growth, and the associated risk of foodborne illness that the product possesses. This research tracked temperature of cut leafy greens in two different systems: first, cut leafy greens served in clamshell containers in school cafeterias, as well as cut leafy greens stored in standard coolers with ice or ice packs to mimic transportation by a farmer to a restaurant.

5.2 Applications

Often industry and policy makers are faced with questions on how to make risk management decisions as well as the impact that these decisions have on food safety. In this example, risk-based decisions are suggested based on the real life temperature data. Gathering real life temperature data is an optimal approach, because average temperatures over several hours can be obtained, and allows for comparison between different systems when determining the best option.

Transporting or storing leafy greens

For leafy greens, the risk of contamination begins at the field. The risk of contamination can be estimated based on on-farm practices, including irrigation systems, likelihood of fecal contamination, and handling behavior of harvest workers. Processing, which typically involves the mixing of multiple batches of leafy greens, is another step at which contamination may occur, further increasing the risk. For industry and policymakers, the risk management decisions made prior to their receipt of the product is primarily not in their control. However, steps could be taken to determine that their leafy greens come from a safe source. This could involve speaking to farmers about their growing practices and encouraging the purchase of leafy greens from farms that are certified in Good Agricultural Practices. This would include visiting the farm to make observations about farm practices, conducting inspections on equipment and facilities, and evaluating the efficacy of current policies. Additionally, microbiological sampling could also be conducted to test for indicator organisms; a sampling plan would allow for frequent checks of what is present in the environment.

Temperature control of leafy greens becomes a primary concern once the processing and cutting step is complete. If leafy greens have been contaminated, temperature abuse after this step can result in proliferation of pathogens. Transporting leafy greens to the buyer may take several hours or longer. As it has been demonstrated in the literature, pathogens such as *E. coli* O157:H7 can grow at temperatures above 5°C. In order to further manage risk beyond

the farm level, farmers and distributors of leafy greens should implement a holding system that consistently maintains leafy greens below 5°C. This could be done through a refrigerated truck or through coolers. An important part to implementing this control is reliability of the system; leafy green temperatures should be checked frequently in order to ensure the system is working properly. The verification system could also be used by vendors at farmers' markets and produce stands, as product may be transported and put out for sale for up to several hours at a time. An effective risk management plan would include this temperature control step lasting from the time the product is cut until it is purchased or consumed.

Results from North Carolina schools suggest that many institutional kitchens may struggle in keeping salads below 5°C during serving. Cooling systems appear to generate a large amount of variation in temperature. This variation is likely due to differences in equipment, particularly how long a system has been in place; systems may decrease in efficiency over time, which could reflect in its ability to stay at low temperatures. Overall, there is a lack of a consistent general standard. This lack of reliability does not allow for effective management of risk at this step; in order to reduce risk, there should be a change in either policy or efficiency of the holding system. Even though the results show that temperatures were above 5°C in many systems, based on Pathogen Modeling Program (PMP) data, temperatures were not high enough for a long enough period to allow for a generation time that was shorter than one serving period, and the lag phase durations for all systems were high (over 20 hours). Therefore, the data shows that in these particular serving systems, it is unlikely that further pathogen growth would occur while leafy greens were on the serving line.

Institutional kitchens may face a challenge in complying with regulations based on cost to replace cooling units, as well as logistics – the current set-up of the kitchen may be limited regarding how salads can be served. However, it would be reasonable to apply a policy similar to time in lieu of temperature (TILT) to all food service kitchens that serve leafy greens, factoring in that some kitchens may take longer to implement this policy based

on cost to make the changes. A similar policy of setting a serving time limitation could be developed to apply to hospitals, schools, and other institutional kitchens, as well as commercial food service systems. The possible time above 5°C would be limited, in turn limiting the ability of bacteria to proliferate. Keeping the allowed maximum temperature set at 5°C would be a good risk management strategy, as it would significantly reduce the amount of time temperatures of leafy greens were allowed to exceed 5°C, reducing the risk of pathogen proliferation through temperature abuse. The policy would work by setting a maximum time point at which leafy greens could be on a serving line, likely up to three hours.

5.3 Conclusions

Although temperatures can easily be tracked during storage of leafy greens, institutional kitchens face a great challenge in measuring temperatures of leafy greens during service. There is currently no system in place to monitor temperatures of leafy greens on the serving line; without this check in place, there is no way to ensure that leafy greens are staying at temperatures low enough to prevent pathogen growth, especially if the leafy greens are being served for long periods of time. Further issues can arise if product is placed back in a cooler after being on the line. The temperature fluctuation between serving line and storage cooler is another concern for institutional kitchens that use a 3-day serving rule. Even if refrigerated temperatures are low enough that pathogens cannot grow, once leafy greens are placed back on the line, pathogens will still be present and have the potential to grow further. However, despite the fact that temperatures reached well above 5°C for the majority of the serving period in some serving systems in this study, the PMP data shows that the serving time is not long enough, or at temperatures high enough, for pathogen growth to occur on the serving line. The PMP data suggests that regulations may not be consistent with actual risk.

Although the results from this project are pilot scale, these policy changes also can be applied to large food service settings that transport and store leafy greens. Control of cut

leafy greens to temperature values low enough to inhibit pathogen growth is critical to effectively managing risk of foodborne illness. Industry and policymakers should frequently evaluate their risk management plan for handling cut leafy greens in order to reduce risk of foodborne illness.

5.4 References

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APPENDIX

Appendix A- *Temperatures of Leafy Greens in Serving Line Systems*

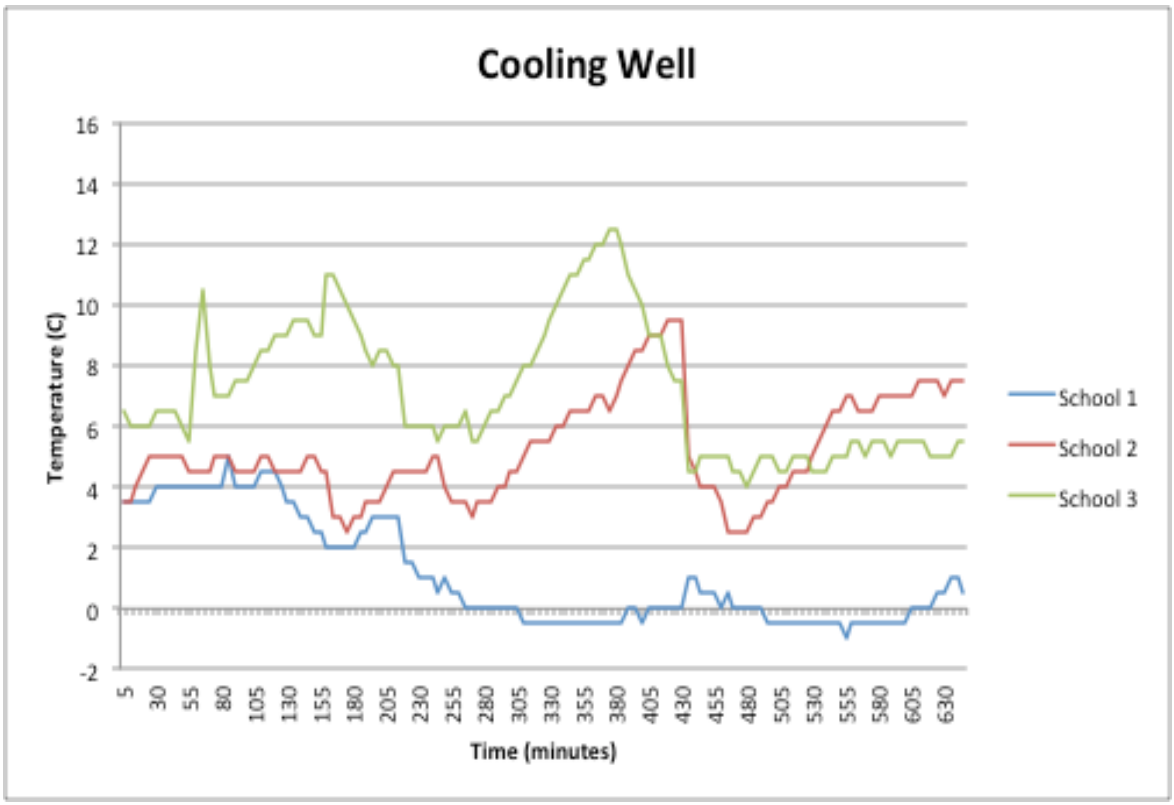

Figure 3.5a. Temperatures of leafy greens held in cooling wells during serving time over 3 days (n = 10). Two schools were TILT. Each time interval is the temperature recorded for each 5 minute period. Day 1 = 1-43, Day 2 = 44-87, Day 3 = 88-131. Standard deviation = 3.03°C (n=10).

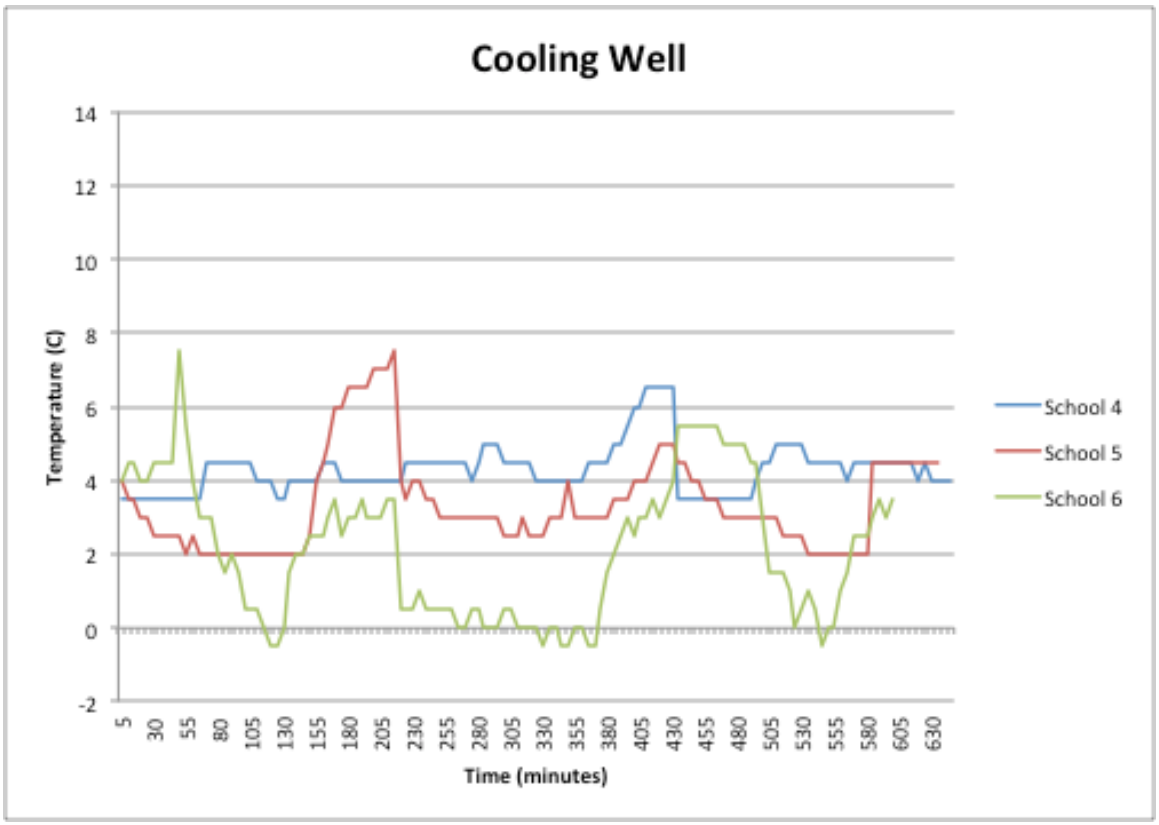

Figure 3.5b. Temperatures of leafy greens held in cooling wells during serving time over 3 days (n = 10). Two schools were TILT. Each time interval is the temperature recorded for each 5 minute period. Day 1 = 1-43, Day 2 = 44-87, Day 3 = 88-131. Standard deviation = 3.03°C (n=10).

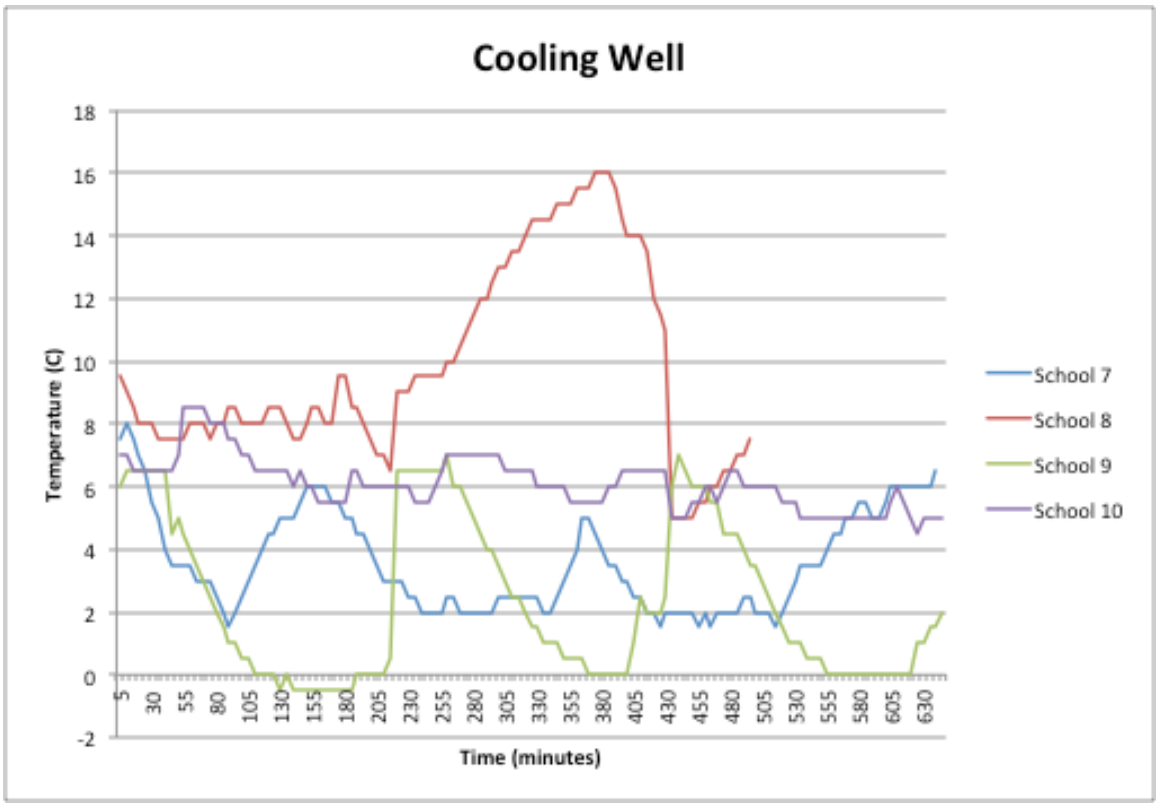

Figure 3.5c. Temperatures of leafy greens held in cooling wells during serving time over 3 days (n = 10). Two schools were TILT. Each time interval is the temperature recorded for each 5 minute period. Day 1 = 1-43, Day 2 = 44-87, Day 3 = 88-131. Gaps occur when measurements were not accurately recorded, or if changes during the lunch periods affected the recording time. Standard deviation = 3.03°C (n=10).

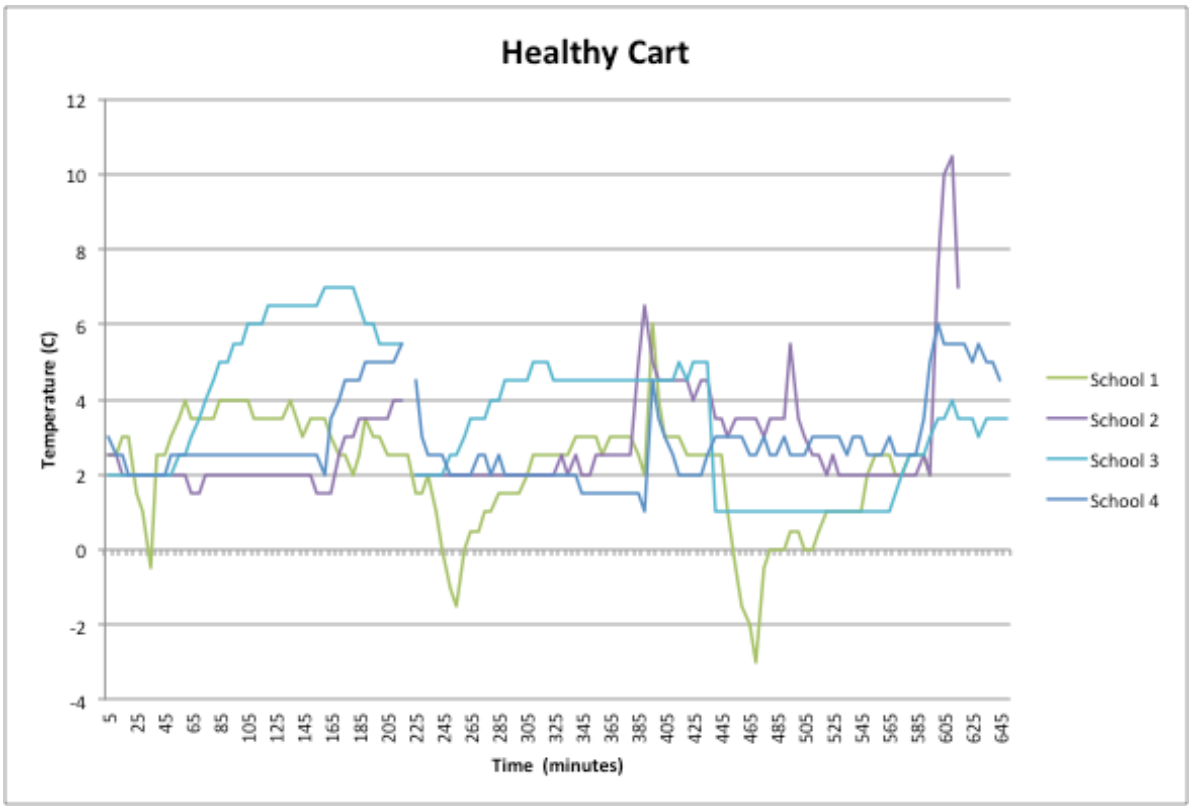

Figure 3.6. Temperatures of leafy greens held in Healthy Carts during serving time over 3 days (n = 4). Each time interval is the temperature recorded for each 5 minute period. Day 1 = 1-44, Day 2 = 45-88, Day 3 = 88-132. Gaps occur when measurements were not accurately recorded, or if changes during the lunch periods affected the recording time. Standard deviation = 1.59°C (n=4).

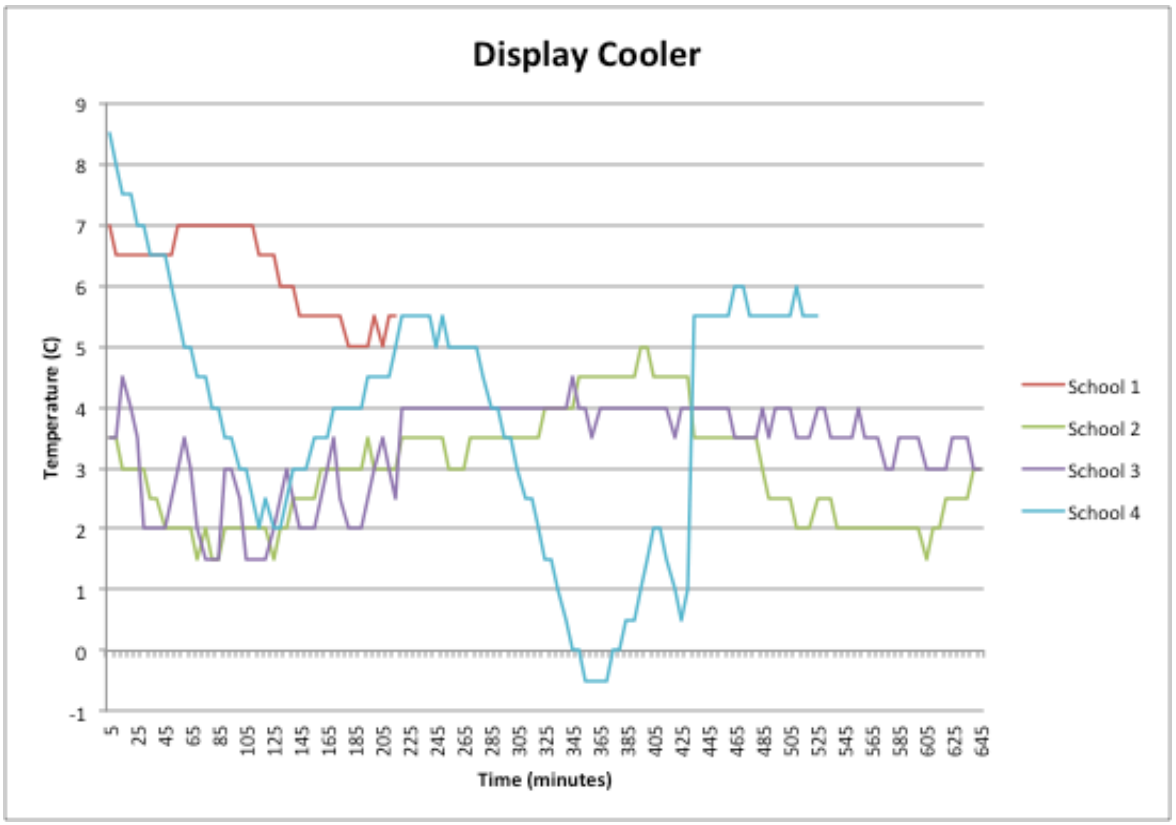

Figure 3.7. Temperatures of leafy greens held in display coolers during serving time over 3 days (n = 4). Two schools were TILT. Each time interval is the temperature recorded for each 5 minute period. Day 1 = 1-43, Day 2 = 44-87, Day 3 = 88-131. Gaps occur when measurements were not accurately recorded, or if changes during the lunch periods affected the recording time. Standard deviation = 1.60°C (n=4).

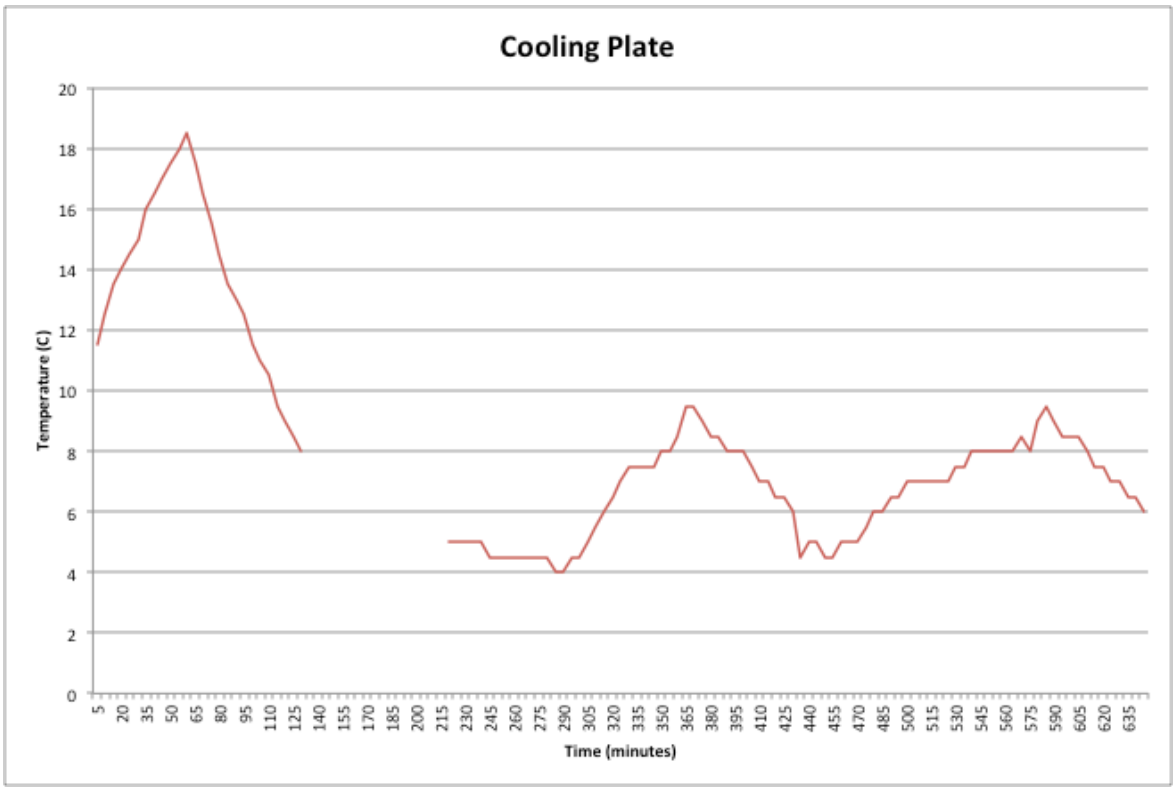

Figure 3.8. Temperatures of leafy greens held on cooling plate during serving time over 3 days (n = 1). Each time interval is the temperature recorded for each 5 minute period. Day 1 = 1-26, Day 2 = 44-87, Day 3 = 88-130. Gaps occur when measurements were not accurately recorded, or if changes during the lunch periods affected the recording time.

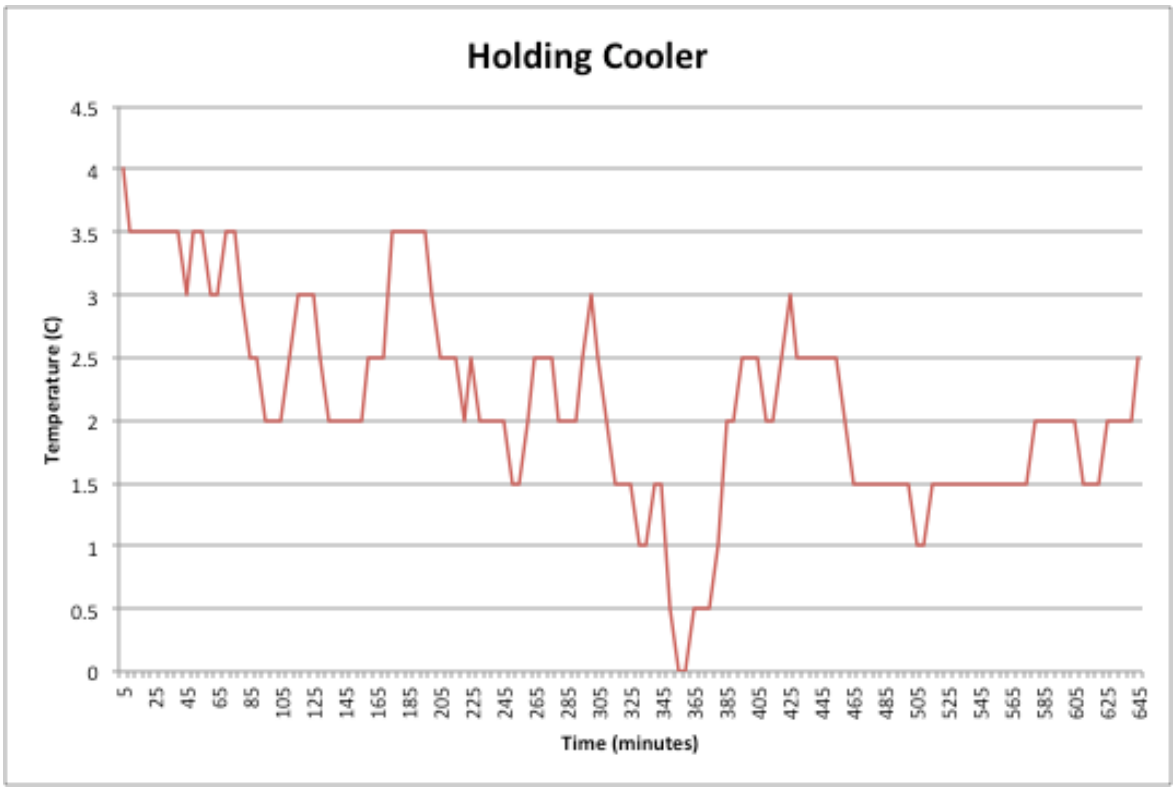

Figure 3.9. Temperatures of leafy greens held in holding cooler during serving time over 3 days (n = 1). Each time interval is the temperature recorded for each 5 minute period. Day 1 = 1-43, Day 2 = 44-87, Day 3 = 88-131.

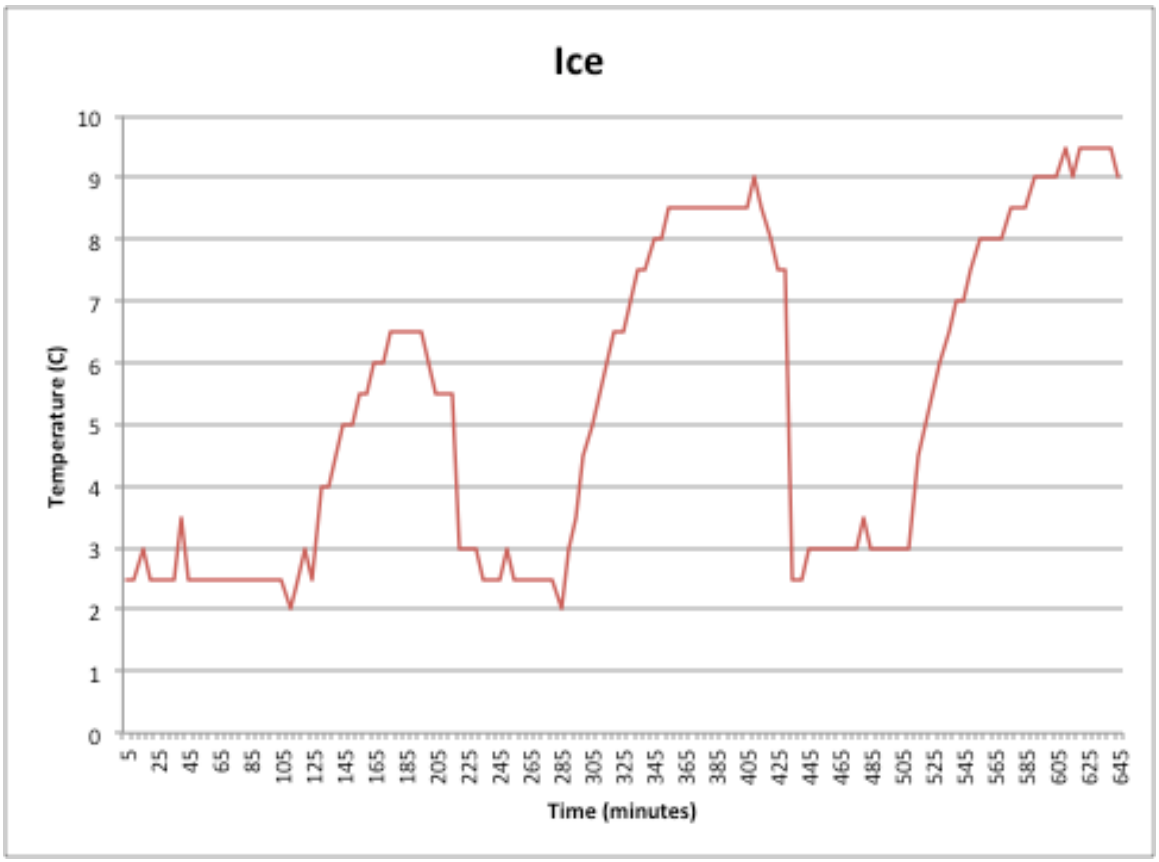

Figure 3.10. Temperatures of leafy greens held on ice during serving time over 3 days (n = 1). This school was TILT. Each time interval is the temperature recorded for each 5 minute period. Day 1 = 1-43, Day 2 = 44-87, Day 3 = 88-131.

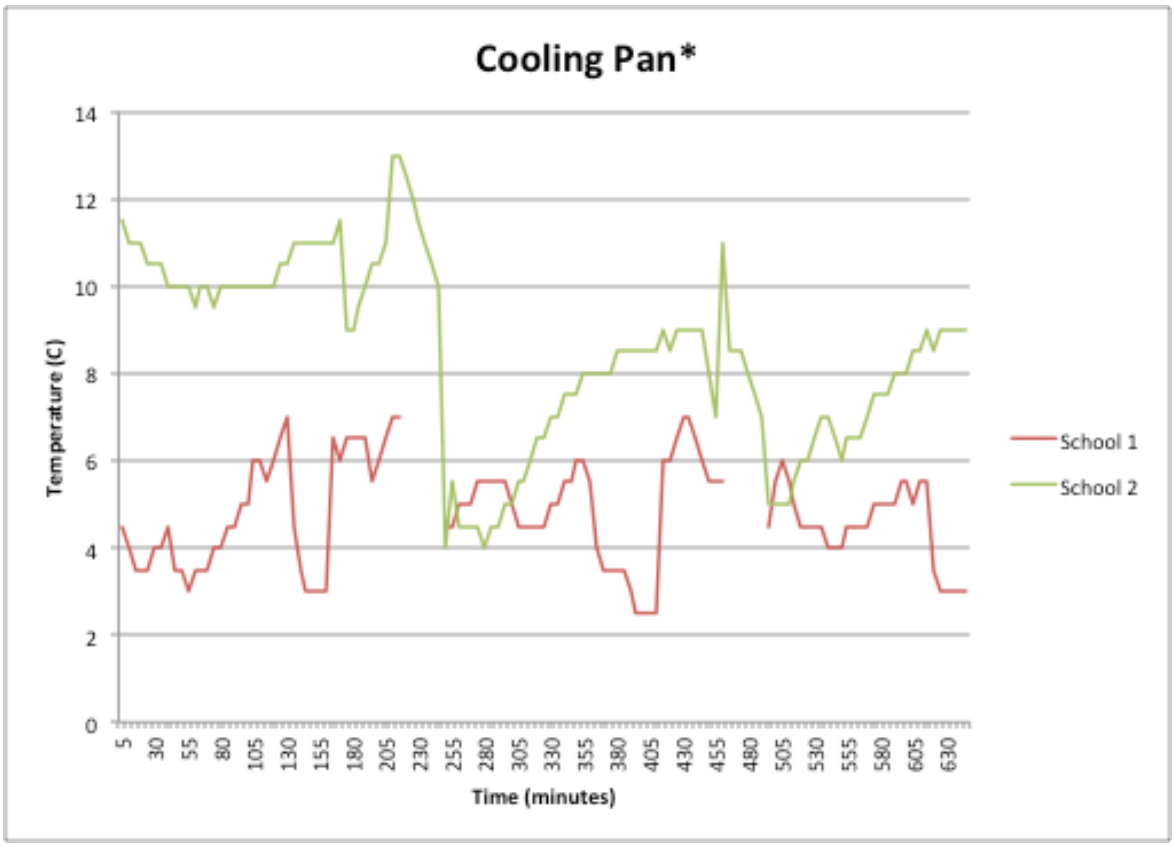

Figure 3.11. Temperatures of leafy greens held in cooling pans during serving time over 3 days (n = 2). The asterisk (*) denotes that leafy greens were served loose in the pan, not in a clamshell container. One school was TILT. Each time interval is the temperature recorded for each 5 minute period. Day 1 = 1-49, Day 2 = 50-99, Day 3 = 100-149. Gaps occur when measurements were not accurately recorded, or if changes during the lunch periods affected the recording time. Standard deviation = 2.49°C (n=2).