ABSTRACT

Alberts, Caitlin Michelle. The Implementation and Evaluation of a Food-Safety Case Study in a Distance Education Course and Pathogen Reduction Using Multiple Hurdle Technique and Encapsulated Nisin During Extended Storage of Shelf Stable Meals Ready to Eat (MRE)(Under the direction of Arritt, F.M., Stevenson, C.D.)

There may be an opportunity to decrease the frequency of foodborne illnesses by focusing on educating employees of food-manufacturing facilities and ensure that food can withstand pathogens throughout its designated shelf life. Therefore, the objectives of this thesis were to reduce the occurrence of foodborne illness by researching (1) the extent to which a multimedia case study of a dairy-processing plant located on campus improves the intentions of students in an introductory Hazard Analysis and Critical Control Points (HACCP) course to implement HACCP upon entering the workforce, and (2) the effect of implementing the multimedia case-study teaching method on knowledge gains and students' satisfaction and determine if pathogens of concern could survive or grow in a shelf stable MRE meat sandwich over the course of a year.

Multimedia case studies were used to enhance student understanding of difficult food safety concepts. The target audience consisted of one hundred percent of participants in an upper-level undergraduate Hazard Analysis and Critical Control Points (HACCP) course (n=18). A pre-test and post-test survey research instrument was developed to measure knowledge gains and students' food-safety intensions using the framework of the Theory of Planned Behavior. Students experienced significant gains in knowledge, attitude and intention after completion of the course ($p < 0.05$). These results suggest that integrating multimedia case studies into food-safety training programs may enhance potential food-

safety behaviors and could, therefore, reduce the occurrence of foodborne illness and recall incidents in the food industry.

The second phase of this thesis focused on a pathogen reduction study on Meals Ready to Eat (MREs). MREs are shelf stable meals used to provide nourishment to military personnel while in the field. To meet the growing demand to sustain these individuals while engaged, individually packed and shelf stable sandwiches have been developed. Nisin was added to the sandwiches in an encapsulated form to inhibit the growth of pathogenic microorganisms of concern over an extended timeframe by slowly releasing the antimicrobial. The MRE sandwiches utilize a multi-hurdle approach as well as the addition of the bacteriocin nisin to limit the growth of pathogenic bacteria, molds and yeasts. Three MRE sandwiches were inoculated with a cocktail of approximately 10^6 CFU/g with a three strain cocktail of *Staphylococcus aureus*, *Salmonella* spp. and *Listeria monocytogenes*, separately. These sandwiches were stored at 26.7°C, 37.7°C and 48.9°C and were sampled over the course of twelve months. The study indicated that the level of pathogens present declined over time and that nisin was active through the duration of the study.

The Implementation and Evaluation of a Food-Safety Case Study in a Distance Education Course and Pathogen Reduction Using Multiple Hurdle Technique and Encapsulated Nisin During Extended Storage of Shelf Stable Meals Ready to Eat (MRE)

by
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DEDICATION

To my parents, fiancé, family and friends.

BIOGRAPHY

Caitlin Alberts was born February 12, 1991 in Wilmington, NC. She attended high school at Eugene Ashely High School and graduated in 2009. Caitlin attended NC State and earned a B.S. degree in Food Science in 2013. After graduation, she decided to continue her education at NC State.

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1.1 Background Information

Foodborne pathogens are responsible for 48 million illnesses, 120,000 hospitalizations and 3,000 deaths annually in the United States, and the associated economic burden is estimated to be $77.7 billion annually (CDC, 2014; Scharff, 2012). Foodborne illness is an ongoing problem in the United States; in fact, some foodborne pathogens are responsible for increasing rates of illness (CDC, 2014; Clayton, 2002). The World Health Organization identified five factors for producing safer food and four of them directly relate to food handlers: improper cooking temperature, temperature abuse, lack of hygiene and sanitation of employees, and cross contamination (Chapman et al. 2010). Enhancing the effectiveness of food-safety training programs should therefore result in a reduction of the amount of foodborne disease.

Effectively training employees who handle food or come in contact with food is necessary to achieve food safety in any foodservice, retail or manufacturing scenario (Egan et. al., 2006). There are not specific guidelines stated by the FDA in the CFR to enhance the effectiveness of food-safety training. Instead, it is up to the management in a processing facility to ensure its workers possesses the appropriate qualifications. Although the information needed to be taught in each training situation differs depending on the processing facility, determining the most effective method to educate the employees is important to ensure that the information is portrayed in best way possible.

To improve food safety competencies in the workplace, University-level students studying food safety should be targeted because many of them enter the workforce as food handlers or supervisors upon graduation. Food-safety managers and supervisors comprised approximately 49,010 personnel in the 2014 workforce (Industries at a Glance, 2015). As the number of jobs continues to increase, the number of educated people available to fill these positions needs to expand because there is a shortage of qualified food safety professionals (Stevenson, 2015). Since these employees have direct contact with employees handling the food, properly training these employees will benefit the entire processing facility as these employees have direct impact on food production employees they supervise.

1.2 Food Safety Training Programs Objectives

An effective food-safety education program must focus on changing the behaviors of the employee or student. Many educational programs solely focus on improving students' competencies such as knowledge and skills; however, improving competencies alone may not always provide the motivation for employees or future employees to practice the subject matter at hand (Egan et. al, 2007). The effectiveness of the training can be more accurately determined by also measuring changes in students' intended behaviors because planned behaviors predict students' performance in applying knowledge upon employment (Egan et al., 2007; Seaman and Eves, 2010).

The skill of applying theoretical knowledge to real-world settings was recently identified as the number-one skill that college undergraduates are lacking upon entering the food industry workforce (Johnson et al., 2014). Knowledge and practice have been

considered separate events for centuries. Chinese philosopher Wang Yang-Ming described knowledge and practice as separated events in the 15th century; stating, knowledge must first be attained before the practice can be put in place (Gallego et al., 2013). While many food-safety education and training programs disseminate knowledge, a key challenge that educators and trainers face is the ability to facilitate practice opportunities for their audience to apply their new knowledge. This is especially a problem for distance education and computer-based training programs. Providing students with training programs that give students the opportunity to apply the knowledge they have learned to a hypothetical and/or real life situation gives students the opportunity to practice. More experience in applying knowledge will improve their ability to preform the behavior (Gallego et al., 2013; Guo, 2012).

1.3 Case Studies

Case studies integrated into education and training programs have been recognized as an effective method for providing students an opportunity to practice and apply their theoretical knowledge. A case study is defined as "a particular instance of something used or analyzed in order to illustrate a thesis or principle" (Merriam-Webster Inc., 2004). Case studies used in education and training programs have been described as "teaching with a story" (Herreid, 2011). Case studies put an educational message into a real-world context, enabling students to better understand examples (Herreid, 2007; Herreid, 1994). The Harvard Business School faculty pioneered case method teaching by integrating business cases into formal classroom activities in the 1920s (Herreid, 2011). Since then, the Harvard Business

School has built a well-respected repository of business cases instructors in higher education to adopt into their teaching activities. The Harvard Business Publishing was established as a nonprofit subsidiary of Harvard in 1994. Harvard Business Publishing has over 350 employees located in the US and abroad to facilitate in bridging the gap between academia and enterprises by providing companies with many different platforms to provide content to those in need. The success of this program provides reason for exploring case method teaching as a means to improve competency gains and behavior changes in food-safety education and training programs (Harvard Business Publishing, 2012).

The underlying principle associated with case studies is that teaching with stories improves learning. Stories help students relate to the content being taught, thus leading students to better understand the information (Abrahamson, 1999). Memorable components of case studies increase students understanding of the subject mater, because they encourage students to remember key details about the story. This enables students to recall more information conveyed through the study (Abrahamson, 1999; Klassen, 2010). The process of teaching with stories enables students to relate to the real world aspects of subject matter, which encourages better understanding of the material.

1.4 Multimedia Cases

Whereas teaching with stories can be done in a variety of ways, multimedia case studies in particular have had positive results. A few of the ways case studies have been taught include lectures, whole class discussions, small groups, individual case instruction and mixed method approaches. These methods typically utilize text-based or lecture methods to teach

the story. Recent advances with multimedia case studies have opened possibilities for making learning more convenient and innovative (Herreid, 2007). For example, Gallego et al. (2013) used a detailed description of a foodborne illness outbreak including photos, a video interview of the people affected to provide students with a case that they must find a solution for. This teaching method is a hybrid approach because the students were provided with face-to-face and online methods. Students positively rated the video case studies, and enjoyed applying the knowledge they learned to real life situations (Gallego et al., 2013).

One key finding of the Gallego et al. (2013) study was that the instructor was an integral part of teaching a case study. It was determined that having an instructor teach the course, although integral, could have some negative implications towards the case study. Some of the instructors participating in the study did not feel comfortable applying the case study to their classroom. Consistency in the way the case study is presented to the students is key. The professor had to be involved in the case study and answer questions appropriately and in a timely manner. Having different instructors teach the same case study could lead to differing results. If a more technologically interactive approach in presenting the case studies was used instead of relying heavily on instructors to facilitate the learning, the rate of computerized feedback is uniform.

Multimedia cases are narratives that are presented in a variety of different media formats including video, audio, interactive and text based methods (McGraw, 2007). The different types of cases have been shown to affect behaviors including the food safety classrooms (Gallego et al., 2013) and other fields (Mach and Janikova, 2010). Although

much time is needed from the instructors to find the right story to use for the case study and create the multimedia case study, many benefits are gained by using multimedia case studies. The use of multimedia case studies benefit the learning process because they are short and to the point, can be emotionally gripping and can summarize events in a concise manner (Pai, 2014). Undergraduate students find traditional lecturing methods in science hard to follow for extended amounts of time, but well-designed multimedia cases can incite interest and curiosity (Wolter, 2013a).

Multimedia case studies have been shown to improve learning outcomes in undergraduate science courses. In a study conducted by Wolter (2013b), 105 undergraduate students in five biology courses were presented in a multimedia case. Students were required to view the case, interpreting the material through tests and sharing the results with fellow classmates through role-playing. They were asked to participate in focus groups and given a pre-test and post-test survey that measured students' confidence in their knowledge of biology. After completion of the learning intervention, students' confidence in their knowledge increased, and they believed that the multimedia cases would help them in their future careers, because they will have already seen how they would apply what they learned to that career. Although knowledge and perception of the multimedia cases were explored, predicted behaviors were not. Employing a measure of predicted behaviors would be beneficial to determine if this method of teaching encourages behavioral change.

1.5 Distance Education Learning

Distance education learning is defined as an instructional setting in which the instructor is separated from the students by time and space (Zapantis, 2008). This type of learning is popular as it provides students with the ability to complete work on their own time, and learn from a remote location (Bender et al., 2010). There are a variety of advantages to distance learning including the fact that students are put in an environment they are able to communicate their thoughts and opinions and generate conversations regardless of time, number of students in the course and pressure from instructors (Darubi et al., 2013; Hew, Cheung, and Ng 2010). Some challenges to distance education learning include instructor resistance to teaching distance education courses, feeling a loss of interpersonal contact, and difficulty providing a hands on experience to the students (Webb et. al, 2005). Many suggest a mixed-methods approach to learning, combining online learning with face-to-face learning (Gallego et al., 2013). When that is not possible, appropriate measures must be taken to ensure students have the best learning interventions when subjected to distance education learning.

1.6 Multimedia Learning

Teaching with serious games has been shown to be beneficial for educating students (Dondlinger, 2009). Serious games are games that utilize the design of games used for entertainment purposes, but are made for educational or training instead (Bergeron, 2006). These games can allow the user to become transported into a learning environment that simulates the real world. (Wattanasoontorn, 2014). Adult students learn best when they can

put their hands on an object, which can be a challenge in a distance education setting (Nakayama and Gin, 2012). Simulating a learning environment is a path toward achieving the hands-on learning approach in this environment. Applying three-dimensional simulations to safety-training scenarios have been shown to be effective at improving learning (Nakayama and Gin, 2012). For example, Guo used game-based technology to provide hands on safety training to construction workers (Guo, 2012). The employees were required to go through the game and a safety messages would emerge if a safety concern occurred. The ability to use game technology allowed employees to train using their own facility, allowing them to become more comfortable with their work environment and learn safety information in real time (Guo, 2012).

Multimedia learning has been used to educating consumers about food safety. Interactive multimedia learning techniques including audio, video, and animation were used to teach infants and mothers food-safety information (Trepka, 2008a). These videos were positively received and participants who had access to the multimedia learning techniques had behavioral changes as compared to the participants that had access only to pamphlets (Trepka, 2008a). This type of learning technique has proven effective in food-safety education and shows promise for possible effectiveness in other studies. (Trepka, 2008b).

1.7 ADDIE Model

The ADDIE model is a recognized framework for systematically improve the instructional design of new and/or existing training programs. The ADDIE model begins with analyzing the objectives of the course (Peterson, 2003). The objectives are determined

early to ensure that future course participants gain something from the course and are done to determine the needs of the course and its audience. Next, the way the instructional information will be designed and provided to the students is determined. The design should be completed before the development process begins. The development phase of the ADDIE model is when the instructional materials are developed. This phase requires time producing and editing materials if videos are to be developed. The implementation phase is when the instructional material is given to the students and put in the course. This phase is when students have access to the learning materials. The final phase of the ADDIE model is when the instructors evaluate the course for improvements and to ensure the course is effective. Using the ADDIE model for course development ensures a continuous model for course development, focusing on improvements to ensure the success of a course.

1.8 Models for Evaluating the Effective Training Program

Evaluating the learning intervention is necessary to ensure that learning intervention used is effective. The way that food-safety competencies are evaluated has been the subject of multiple studies. In a review conducted by Soon et al. (2012), nine studies that focused on different training interventions and measuring employees' knowledge and attitudes. It was concluded that studies that used cognitive-behavioral theory-based models in in conjunction with standard training to evaluate their programs had a greater final hand-washing rate as compared to studies that did not use the theory.

Following a model for evaluation is necessary to ensure that the evaluation is done correctly (Viator, 2015). There are a variety of models that function under the assumption

that beliefs, attitudes and norms determine a persons' behavior and use of a specific model is determined based on the social and environmental settings the model will be applied under. Different models have been used to examine food handlers' behaviors as well as better understand consumer behaviors including the theory of planned behavior, health belief model and the SPARTA model (Clayton, 2002; (Egan et al., 2007; Mullan and Wong, 2009; Vainio, 2013).

The subjective norm, perceived behavioral control, attitude, risk, trust and alia (other variables) model, commonly referred to as the SPARTA model, is a model used to measure planned behaviors. It assesses risk and trust and how these variables affect behavior (Lobb et al., 2007). The SPARTA model has been applied in a study that analyzed if consumer's trust of the food industry chose to eat chicken as well as a study that determined if climate change played a role in food choice (Lobb et al., 2007; Vainio, 2013).

Although the SPARTA model has been used within the food industry, it should be used when trust or risk is a factor in performing a behavior. Trust refers to the trust of food safety information given from the media, scientists, family, etc. When using a model for food safety education, trust is not appropriate because the employees are learning what they should practice in their chosen profession.

The health-belief model is a commonly used model in the health industry and is used to predict behaviors regarding health (Hayden, 2009). This model uses susceptibility, seriousness, benefits and barriers to explain health behavior. This model has been used in a variety of studies including a study determining the effectiveness of nutritional information

provided to diabetics (Sharifirad, 2009). Although it is commonly used in the health industry and has been used to measure food-hygiene behaviors, it was found that the Health Belief Model was a poor predictor in undergraduate students and it does not target normative behaviors, social pressures that influence choices impacting behavior (Mullan and Wong, 2009).

1.9 Theory of Planned Behavior

The Theory of Planned Behavior (TPB) model is used to predict behavioral intentions (Figure 5). The TPB was built to improve the theory of reasoned action, which was a theory also developed by Ajzen utilizing behavioral beliefs and subjective norms to predict behavioral intention (Ajzen, 2006). The TPB utilizes the theory of reasoned action, but also incorporates the variable of perceived control beliefs (Madden, Ellen and Ajzen, 1992). The addition of the perceived control beliefs enhances in the prediction of behavioral intent and behavior (Madden, Ellen and Ajzen, 1992).

The TPB explains that behaviors, and behavioral intent are influenced by three variables. The behavioral beliefs variable describes the feelings or attitude a person has associated with a particular behavior (Ajzen, 2002). These include the positive or negative feelings about the performing an action. For example, "performing a certain behavior would make me feel x" (Orbeil et al., 1997). This can be evaluated by using an attitudes scale. The perceived behavioral control is another variable used to predict intentions for the TPB. This variable measures the confidence that one can act including the ease or difficulty one feels for preforming the necessary behaviors (Ajzen, 2002). The easier someone perceives a behavior

to be, the more likely they are to perform that behavior. The perceived behavioral control can be measured by using four different questions, two for controllability and two for self-efficacy (Mullan and Wong, 2009). The third variable is subjective norms. This variable determines how one feels based on social pressures from others (Ajzen, 2002). This includes the influences from friends, teachers, bosses, etc. The greater the social pressure to perform a behavior, the more likely it is for someone to perform the behavior.

The three variables (behavioral beliefs, subjective norms and perceived behavioral control) all influence the behavioral intent (Orbeil et al., 1997). The greater the person's subjective norms, behavioral beliefs and perceived behavioral control, the more likely one intends to practice a behavior. If one has a great amount of perceived behavioral control, it directly affects the person has a greater intent to perform the behavior (Orbeil et al., 1997). Intention is described as the motivational orientation of toward a behavior (Ajzen 1991).

The theory of planned behavior has been shown to be effective with health-related behaviors and risky behaviors. The TPB has been used in a variety of food safety related studies to predict behavior including studies targeting safe-food handling practices in the home, and workplace (Egan et al., 2007, Shapiro et al., 2011). In a study conducted by Shapiro et al. (2011), the theory of planned behavior was used to learn about consumers' adoption of food-safety behaviors in the home. Roberts et al. (2008) used the theory of planned behavior in 31 restaurants to determine whether employees would make a behavioral change after completion of a training program. In both studies, the theory of planned behavior aided in determining whether participants would perform a food-safety behavior.

In an educational-based study with the objective of determining if participants would practice a food-safety behavior, this model would be a well-designed one to use.

1.10 Summary

Effectively educating college students who are likely to join the food-safety workforce on current food safety programs will supply the food industry with qualified employees. In order to properly educate the students, the education program needs to focus on changing behaviors as well as enhancing food safety competencies. Case studies have been shown to influence behavioral changes in face-to-face studies and providing distance education courses with multimedia case studies has potential to improve students' food safety behaviors and competencies.

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CHAPTER 2: The Implementation and Evaluation of a Food-Safety Case Study in a Distance Education Course

Abstract

There is opportunity to decrease the frequency of foodborne illnesses by improving food-safety competencies and behaviors of workers throughout the supply chain. Therefore, the objectives of food-safety training programs should include efforts to improve students' behaviors and increase achieving knowledge gains. The skill that food-safety undergraduate students lack the most after graduating is the ability to apply what they have learned to real-life applications. Multimedia case studies were used to enhance student understanding of difficult food safety concepts. The objectives of this study were to evaluate (1) the extent to which a multimedia case study of a dairy-processing plant located on campus improves the intentions of students in an introductory Hazard Analysis and Critical Control Points (HACCP) course to implement HACCP upon entering the workforce, and (2) the effect of implementing the multimedia case-study teaching method on knowledge gains and students' abilities to understand complex concepts.

Central to the case study was a documentary video that portrayed a typical work day in a dairy manufacturing facility, a video that gave students a tour of the facility, and an interactive blueprint of the facility containing hotspots that played videos showing examples of Good Manufacturing Practices in various locations. Students interacted with these videos through weekly discussion forums, time-linked questions that appear while watching the videos, and a course project of developing a HACCP plan for a product processed at the

facility. The target audience consisted of one hundred percent of participants in an upper-level undergraduate Hazard Analysis and Critical Control Points (HACCP) course (n=18). A pre-test and post-test survey research instrument was developed to measure knowledge gains and also students' food-safety intensions using the framework of the Theory of Planned Behavior.

Students experienced significant gains in knowledge, attitude and intention after completion of the course ($p < 0.05$). One hundred percent of students agreed to some extent that the interactive videos aided in their understanding of food-safety concepts. A paired t-test comparing students' pre-test and post-test survey responses suggested that, unlike normative beliefs ($p > 0.5$), the control beliefs ($p < 0.5$) and behavioral beliefs ($p < 0.5$) and intention ($p < 0.5$) were significantly changed after completion of the HACCP course. These results suggest that integrating multimedia case studies into food-safety training programs may enhance food-safety behaviors and could, therefore, reduce the occurrence of foodborne illness and recall incidents in the food industry.

2.1 Introduction

Foodborne pathogens are responsible for 48 million illnesses, 120,000 hospitalizations and 3,000 deaths annually in the United States, and the associated economic burden is estimated to be $77.7 billion annually (CDC, 2014; Scharff, 2012). Many outbreak and recall events are a result of post-process contamination or poor personal hygiene (Neal and Crandall, 2014), which are preventable through ensuring the workforce possess appropriate behaviors and competencies (Chapman et al., 2010). While many food-safety training interventions reported in the literature have focused on food-service and retail applications (Viator, 2015), interventions for food manufacturing environments have been less commonly reported (Sperber, 2005).

Better preparing college students to enter the industry should supply the workforce with more qualified employees and improve food safety in manufacturing facilities. All employees of a food manufacturing facility should be trained in food safety, especially food-safety supervisors and managers because of the leadership responsibilities their job entails and because of a shortage of qualified professionals (Freudenheim 2009; Scott-Thomas 2012; Stevenson, 2015). There is a need to continuously improve food safety curricula in higher education because college graduates are often hired into supervisory positions in food-manufacturing facilities (National Research Council, 2009).

Successful implementation of food-safety management systems is a critical job task for managers in food manufacturing. HACCP is a systematic approach to reduce foodborne illnesses by identifying the hazard and controlling the hazard through prerequisite programs

and critical control points (Wallace, 2014). This system of controlling food safety is globally recognized and meat and poultry processing facilities have been required to implement a HACCP plan since 1993 (Pearson and Dutson, 2012). HACCP is recognized by international organizations including the Codex Alimentarius and is required to be implemented in facilities that manufacture meat (9 CFR 417, 2015), poultry (9 CFR 417, 2015), juice (21CFR 120.8, 2015), and fish and fishery products (21 CFR 123.6, 2015). Manufacturers of Grade A dairy products have the option to implement a voluntary HACCP plan to comply with the Pasteurized Milk Ordinance (FDA, 2014).

Despite the resources and efforts invested into implementing beneficial training programs for food manufacturing facilities, there has not been a significant decrease in foodborne illnesses (Neal and Crandall, 2014; Viator, 2015). One explanation is that a majority of education programs do not show evidence of food-safety programs being effective, and instead focus on knowledge gains (Viator, 2015). Although knowledge gain is important, targeting a specific behavior, attitude and aspiration is the key to ensuring the desired behavioral change will actually be achieved (Egan et. al, 2007; Low et al., 2013; Seaman and Eves, 2010). A HACCP course that successfully focuses on the behavioral changes of the participants is needed to decrease foodborne illnesses in food manufacturing facilities in the United States.

An effective evaluation program is necessary to demonstrate the impacts of food safety education and training programs (Wallace, 2014). In a review of food safety training programs conducted by Viator et al. (2015), only three out of 23 cases adopted a framework

or theory for their program evaluation. To ensure participants' food-safety behavioral intentions are impacted by a training program, an approach for evaluating food-safety education and training programs' impacts on planned behaviors is needed. The theory of planned behavior is founded on the premise that social pressure, attitude towards a behavior, and perceived control over performing the behavior predicts a person's intent on preforming the behavior (Figure 5)(Armitage and Conner, 2001). Behavior is influenced by intentions and the perceived control one has over performing the behavior (Azjen, 1991). Intentions are described as how willing and how much work someone would put into performing a behavior (Azjen, 1981). By providing a training program that targets the factors affecting intentions and thus behavior, the training program is more likely to influence participants' future behaviors. The theory of planned behavior has been used to predict behavioral intention of students learning to drive (Ferguson, 2005), and athletes (Palmer et al., 2005) (Egan et al., 2007; Roberts et al., 2008; Shapiro et al., 2011).

The case-study method of teaching can improve food-safety competencies and behaviors (Yiannas, 2015). A case study is defined as "a particular instance of something used or analyzed in order to illustrate a thesis or principle" (Merriam-Webster Inc., 2004). These learning interventions can be presented in a variety of ways, including discussions, group work, lectures or combinations of these (Abrahamson, 1999; Herried, 2007; Pai, 2014). Case studies can provide students hands-on experiences in applying theoretical knowledge to a specific scenario that has real-world context. Hands-on experience is not always achieved in college science courses, particularly in a distance-education format. (Gallego et al., 2013). Providing students the opportunity to apply theoretical knowledge

toward a case study gives them a valuable experience that will improve their preparedness for their future careers (Wolter, 2013).

The addition of videos to case studies enhances learning and student engagement, which can improve desired behavioral changes (Pai, 2014). Both traditional college students and non-traditional students tend to learn best in environments where they have hands-on experiences (Cantor, 1997; Nakayama and Jin, 2015). When the facilitation of hands-on learning is not feasible, one option is to integrate videos into a case study such that students can virtually experience and conceptualize the case. This has been shown to improve student engagement and understanding (Pai, 2014). Adding an interactive component to the case study also aids comprehension and has been effective in a variety of other safety-related fields (Guo, 2014).

The objectives of this study were to adapt the theory of planned behavior to measure the behavioral changes of the participants in a food-safety distance education course and determine to what extent a multimedia case study can assist students' abilities to apply theoretical knowledge of HACCP to real-world scenarios.

2.2 Rationale and Significance

The purpose of the study was to find a more effective way to educate individuals on food safety. If potential food-safety employees were given more productive training, and were taught to be alert to problems they could prevent in the workplace, the employees would be better equipped to handle the needs of their job and lower the amount of food-safety risk. To achieve this, knowledge gain as well as behavioral change should be targeted. Focusing on targeting participants' planned behaviors and ability to apply what they have learned will

ensure that what is learned in the classroom will actually be applied to the day-to-day life of the employee's job.

The authors of this study hope that the information gathered will be used to improve training programs in the food industry. Currently, the majority of food-safety training programs in the food industry focus on improving the knowledge of their employees, but this does not always transfer into the employees' behaviors. By developing a training program that focuses on changing the behaviors of students and developing a rubric for making the tool, this study will have a positive impact on the educational section and eventually the workforce in the food industry.

2.3 Experimental plan

The analyze-design-develop-implement-evaluate (ADDIE) model was used to frame a course redesign project for a senior-level introductory HACCP undergraduate course, (Peterson, 2003). The course was a distance-education course taught through the Moodle Learning Management System (LMS) (Learn N.C., 2009). The ADDIE model followed a linear view of course development, encouraging constant revisions and improvements. It is based on the understanding that learning should be student-centered, innovative, authentic and inspirational and is a common model used for performance-based learning (Branch, 2010).

2.4 The ADDIE Model

The ADDIE model consists of five phases to target each step in course development. The analyze phase was used to determine the learning needs of the students, the

competencies their employers are looking for, and the constraints and resources available for the course redesign project. This was done to identify gaps the case study is to fulfill. (Branch, 2010). The design phase identified how to accomplish the objectives (Branch 2010). The development phase of the ADDIE model entailed the creation of the learning interventions. The implementation phase involved teaching the course throughout a 16-week semester, which was assessed during the evaluation phase. By completing each phase, a continuous process for course development and improvement was achieved.

2.5 Analyze Phase

The objectives for the case study were determined by compiling information gathered from past class evaluations, subject matter experts, instructional designers, and a review of the literature. A team of subject-matter experts reviewed students' feedback from previous semesters and chose commonly reoccurring problems and suggestions provided by the students. For example, previous class evaluations indicated students thought the instructor needed to better explain difficult concepts. The majority of students in the introductory HACCP course had never been inside a food manufacturing facility. Teaching how a food manufacturing facility works through distance education is a challenge because students have a basic understanding of food manufacturing before they can be expected to build a HACCP plan.

In a recent study conducted by Johnston et al. (2014), it was discovered that applying theoretical knowledge to real-world settings was the number one skill college graduates lack (Johnston et al., 2014). Even though a student may know the information, the student may

not know how to apply this in the workplace or other situations (Egan et al., 2007). By providing students with a case study of a food manufacturing facility, they are able to take the information from the course and apply it directly to a simulated version of a work environment. This gives students the ability to practice skills that they are expected to apply once accepting a position in the food-safety field.

2.6 Design Phase

To achieve the objectives determined in the analyze phase, the course needed to be re-designed with the objectives of preparing students to (1) explain difficult material well, and (2) apply theoretical knowledge after completion of the course. Teaching with a story is expected to increase student understanding and their planned behaviors (Abrahamson, 1999). Case studies enhance engagement, memory and help students apply what they have learned to simulations of the real world. Therefore, the case-study approach was chosen because of its capacity for students to apply what they have learned to real-world situations and break down a complicated situation in a way that makes the student feel part of the situation.

The case study was based on the Howling Cow™ dairy plant, which is located on campus at North Carolina State University. Howling Cow™ processes Grade A milk and ice cream products that are sold on campus and at the North Carolina State Fair. The Howling Cow™ operation is a vertically integrated business.

Videos were chosen as the format for the case study because videos have been shown to create a higher level of engagement for the participants and create an emotional connection to the content (Yadav, 2011). The case study consisted of a series of three videos detailing the Howling Cow™ process and regulations and 12 videos highlighting Good Manufacturing

Practices (GMPs) in different locations inside the processing facility (Stevenson, 2014a; Stevenson, 2014b; Stevenson, 2014c; Stevenson 2014d).

The three videos detailing Howling Cow™ were produced to build a connection between the student and the processing facility such that many teachable moments could be highlighted throughout the course. Videos have the ability to create an emotional connection between the characters and the person viewing the video (Borup, 2012). Emotional connections stimulate learning and allow for value and meaning to be involved in the learning process improving and expanding the learning implications (Yadav, 2011). The objective of the "Ice Cream Makers" video was to give students an idea of what a day in the life of a worker in the Howling Cow™ dairy processing facility is like. It was designed to help students develop an emotional connection between the viewers and the employees at the plant. "The Processing Tour" video was a walk-through of the dairy plant and taught the students how the ice cream is made, what equipment is used, and how the product flows through the plant. The objective of this video was to teach students how the dairy plant works and provide information in a way that is easy to understand. The "Pasteurized Milk Ordinance vs. HACCP" video was designed to give an understanding of how food-safety regulations impact the managers of the Howling Cow™ dairy processing facility. Providing the information in video form was done to engage the viewer while information being taught, encouraging learning.

Twelve videos were designed to teach students how to look for problems with GMPs in specific sectors Howling Cow™ facility. These videos highlighted twelve locations in the processing facility and two employees from Howling Cow™ spoke on camera.

The plant tour GMP game was designed to give students an interactive approach to learning. The objective of this game was to help the students apply the information that they have learned to a real life situation that would mimic a possible future job and give them virtual experience in Howling Cow™. The twelve GMP videos were inserted into a blueprint of the Howling Cow™ facility. Students were required to click through the blueprint, and watch one of the twelve videos. Once the video was completed, an assessment popped up to determine if students could pick out the Good Manufacturing Practices present in the video. The student's score would then be calculated and displayed on the blueprint.

2.7 Development Phase

A collaborative team consisting of an instructional designer, video production manager, two video production specialists, subject matter expert, and a teaching assistant produced the 12 GMP tutorial videos, the processing tour, day-in-the-life and PMO vs. HACCP video. It took approximately two weeks to record footage for all the videos and another two weeks to edit rough cuts, each of which received multiple revisions.

The twelve GMP tutorial videos were developed over the course of two days. These vides ranged from 39 seconds to two minutes and 12 seconds. The "Ice Cream Makers" video which covered the process from start to finish and employees was 11 minutes and 12 seconds long (Stevenson, 2014a). The processing tour video was six minutes and thirty-four seconds (Stevenson, 2014b) The PMO vs. HACCP video was five minutes and thirty-two seconds (Stevenson, 2014c). A blueprint of the facility was created and hotspots were added to the locations where the GMP videos were produced. Clicking on the hotspots played the videos and students were required to answer questions at the end of the videos.

2.8 Implementation Phase

The Howling Cow case study was implemented into the course in multiple ways to ensure that the participants had continuous exposure to the case study. Throughout the 16-week course, the case study was implemented into the course in a variety of ways, including knowledge assessments, discussion forum posts and responses, a Moodle workshop activity and an interactive game.

Students were given a knowledge assessment in the form of a quiz. The quizzes were timed for 20 minutes and ranged between 5 and 10 multiple-choice, true/false or fill-in-the-blank questions. The weekly discussion forums required students to answer a question posed by the instructor and then reply to a fellow student's response. This discussion method is beneficial in distance-education courses by improving higher cognitive thinking (Sautter, 2007). The instructor provided students with resources to be used, and then posed a question for the class to encourage discussion on the topic. For the majority of the discussion forums, students were instructed to watch a specific Howling Cow documentary video. After watching the video assigned for the week, the students were instructed to submit an initial post answering a specific question relating the documentary video to the week's lesson content. After their initial submission, students were required to reply to another student's post. This was done to enhance discussion and facilitate community in the class.

Throughout the course, students developed a HACCP plan based on a Howling Cow^{TM} product. Each student was assigned a different product and added to their HACCP plan each week in relation to the week's lesson content. This assignment enabled students to

have a hands-on approach to developing a HACCP plan for a company with which they were involved. Once the student submitted their updated HACCP plan, they were assigned to review two of their classmates' work and grade their work following a rubric developed by Wallace et. al (2005). This was done to facilitate community in the course, and give students the opportunity to view other assignments and continuously improve their own.

Students were required to watch the "Ice Cream Makers", "Pasteurized Milk Ordinance vs. HACCP" and "Processing Tour" and complete time-linked questions facilitated through EduCanon (eduCanon, 2014). Throughout the video, different questions related to the videos popped up. Students had to answer the question correctly before they were able to continue watching the video. In order to earn credit for watching the video, students had to provide the code listed at the end. This encouraged students to watch the full documentary video and allowed the videos to have an interactive component. The interactive plant tour GMP game component used was a required activity during the weekly lessons on prerequisite programs in food manufacturing.

2.9 Evaluation Phase

To evaluate the effectiveness the video case studies had on the distance-education students, a pre/posttest survey was developed and distributed to a treatment group (Figure 18). The treatment group for this study consisted of an upper level introductory on HACCP (n=18). The survey instrument was used for both formative and enhancement purposes and was distributed with a 100% response rate.

2.10 Model Integration

Survey questions were developed to determine to what extent difficult information was taught. These questions included how the instructor explained information, and what aided in the student's understanding of the course information. The framework for the theory of planned behavior was used to determine if objective two was achieved. Questions regarding student's normative beliefs, behavioral beliefs, control beliefs and intention were developed and distributed.

2.11 Survey Development

The pretest survey for the food-safety course contained 66 different questions. This study consisted of demographic questions, four aspiration questions, 10 knowledge questions, four questions on control, six normative belief questions, six risk questions, five attitude questions, five open-ended questions in regards to belief and 11 attitude questions. The post-test survey for both courses included all of these questions along with formative questions to be used for course improvement.

The reliability of the survey was justified by using questions obtained from surveys. Surveys using the theory of planned behavior in similar fields were compiled using a literature review and the questions were modified to address food-safety questions (Brook, 2006; Gallego et al., 2013; Morgan et al., Pragle et al., 2007). Cronbach's alpha was calculated for each likert section of the survey. Each likert question received a rating of 0.70 or higher (Table 8) (Knabe, 2013).

The validity of the survey was conducted by working with experts and students alike.

Before the distribution of the survey, a delphi style method was used to improve the content validity of the survey in which the survey was provided to three experts in the field (Duke, 2009). Their suggestions were documented and changes were made. The survey was then sent to the experts again to discuss the revisions made. Two focus groups of three students each were used before survey distribution to improve face validity of the survey. The survey was provided to groups of students and discussed at length to ensure that each question was worded in an appropriate way and to ensure the students understood what was being asked in each question.

2.12 Survey Distribution

The survey was developed using Qualtrics (Qualtrics, USA), approved by an institutional review board (IRB) and distributed to the students through the Moodle Learning Management System. The students were required to take the survey as part of a grade. The pre-test and post-test survey contributed to a total of 2% of the course grade. Students were provided with the option to withhold their information from being used in the research and still earn 100% of the allotted course grade. This survey took students approximately 30-40 minutes to complete.

2.13 Statistical Analysis

The data for this study was analyzed using Qualtrics and SPSS AMOS (Wuensch, 2014). A pathways analysis was conducted to discover to what extent specific variables influenced intention. The variables selected to study were normative beliefs, control beliefs and behavioral beliefs. The variables were all apart of the theoretical framework of the theory

of planned behavior. The pre-test and post-test data were compared using paired t-tests to determine to what extent the learning intervention significantly changed the variables being studied (95% confidence interval). The post-test data were also analyzed to determine the demographics gathered from the study.

2.14 Results and Discussion: Objective 1

Objective 1: Determine the successes and failures of teaching with an interactive case study

Explaining difficult material well.

When asked to what extent students' thought the instructor explained difficult material well, students rated the instructor 4.2 on a 5-point scale and a standard deviation of 1.0 (Figure 1). This suggests that the students believe that the instructor explained the information well. In the previous semester, the students rated the instructor with an average score of 2.7 and a standard deviation of 1.4. Although the classes were not the same, the difficult material was explained significantly better than in previous semesters ($p < 0.05$). The learning interventions used in the improved Introductory to HACCP Course focused on providing students with information in a manner that engaged the student as well as informed the student on the subject matter at hand. Being able to see the processing facility gave students a better idea of the environment that was being discussed. The interactivity of the game based learning modules allowed students to apply what they had learned to a real life situation. This finding is consistent with other studies that have concluded that videos make difficult concepts easier to understand (Mehrpour, 2013; Shiatis and Tsiligiannis, 2013).

Application of theoretical knowledge

One hundred percent of students believed that the "Ice Cream Makers" video aided to some extent in their understanding of difficult concepts (Figure 2). One hundred percent of students believed that the "Processing Tour" video aided in their understanding to some extent, as well. The "PMO vs. HACCP" video was analyzed to determine whether the students' knowledge of PMO and HACCP was improved due to the video. Ninety-four percent of students self-reported that the PMO video aided in their understanding, and only six percent of students neither agreed nor disagreed that the video helped. None of the students disagreed with the statement that the videos helped understanding overall. This suggests that the students believe that the videos helped their understanding of topics.

Knowledge Improvement

This course significantly increased the knowledge of the participants between the pre-test and post-test (Figure 3). This is consistent with numerous studies conducted in the health industry where videos were used to improve the viewer's knowledge on a health-related subject (Brace et al, 2010; Del Carmen Cabesa, 2014; Trinh et. al, 2014). The entirety of knowledge gains cannot be attributed to the videos, but the videos likely contributed to knowledge gains to some extent.

2.15 Results and Discussion: Objective 2

Adapt the theory of planned behavior to measure the behavioral changes of the participants in a food-safety distance education course.

A pathways analysis was conducted to determine the fit between the TPB model and

the data recovered from the post-test survey (Figure 4). The pathways analysis suggested that the behavioral beliefs variable has the greatest affect on intention to perform a behavior, thus making this variable one that should be targeted at a greater rate. In the pathways analysis, behavioral beliefs ($p < 0.1$) and control beliefs ($p< 0.1$) had a significant influence on student's intention to implement HACCP in their careers. This suggests that behavior beliefs and control beliefs are important factors when determining the food safety behavioral intent students; behavioral beliefs having the strongest correlation.

Normative beliefs, or social pressures one feels about a particular behavior, did not have a significant effect on influencing intention ($p > 0.1$). In a study conducted by Rigby et al. (2013), athletic trainers opinions regarding concussion management practices were examined using the TPB. Subjective norms did not significantly predict the athletic trainer's behavioral intent. This study suggested that the subjective norms should be used for managing compliance with practicing behaviors and the intervention should be focused on changing the attitude of the participant instead of social aspects. Ingram et al. (2000) found similar results when analyzing students' intentions to applying to graduate school. A pathways analysis was conducted and found behavioral beliefs to have the strongest correlation to intent and subjective norms to have the weakest correlation. It was hypothesized that subjective norms had the weakest correlation due to the self-determination theory, which states that people who set goals for themselves are more persistent with their goals because social encouragement can be seen as added pressure (Ingram et. al, 2000). This pathways analysis suggests that focusing a learning intervention on improving the behavioral beliefs of participants will lead to a greater rate of behavioral intention.

A paired t-test was conducted to determine the changes between pre-test and post-test survey scores (Table 9). There was a significant change in the student's behavioral beliefs between the pre-test and post-test after completion of the course (Figure 8). There was not a significant change in the control beliefs and normative beliefs (Figure 6-7). Subjective norm had the weakest influence on behavioral intent, which is consistent with findings stating that subjective norm does not have a strong influence on intent, especially as compared to perceived behavioral controls and behavioral beliefs (Rigby et al., 2013). The course could utilize technology to allow more social interaction in the course. There was a significant change when students were asked about the social pressures felt by their teacher. Students significantly believed that the teacher added pressure for them to perform positive food-safety behaviors ($p < 0.05$; Figure 7). This suggests that the person giving the training program or in charge of the learning has a greater effect on the student's preforming the learned behaviors well.

The student's control beliefs, or feeling that they had control over performing and controlling food-safety behaviors, did not significantly ($p > 0.05$; Figure 6) improve after the learning intervention. Students learned how to develop a HACCP plan, but not necessarily manage a HACCP plan. Many of the students (96%) were not employed in the food industry at the time they took the course, so perhaps their lack of work experience inhibited them from imagining themselves managing and controlling the HACCP plan once in the industry. Adult students learn better with hands-on approaches, and enhancing the opportunity for students to receive a hands-on approach aids in learning and may improve control beliefs (Nakayama and Jin, 2015).

There was a significant improvement in the behavioral beliefs of students, or attitude toward the behavior and their intention to perform the behavior ($p < 0.05$) (Figure 8). As stated previously, focusing a learning intervention on improving behavioral beliefs will lead to greater rate of behavioral intention. The learning intervention preformed in this study significantly improved the behavioral beliefs, suggesting that the course provided students with a positive outlook on food-safety and the need for food safety. Azjen (2007) stated that in order to increase an attitude regarding a behavior, the perspective of that behavior should be addressed in the learning intervention. The learning intervention proposed in this study uses a multimedia case study to influence behavioral beliefs. The significant improvements to participants' behavioral beliefs after completion of the study highlight the positive effects of the multimedia case study and thus encourage students to implement HACCP plans in their future facilities.

2.16 Limitations and Potential Pitfalls

A limitation of the study is that a control group was not compared to the treatment group which could isolate a more exclusive analysis of the effect of the multimedia case study. Another limitation to this study was the sample size. The course consisted of only 17 students due to limited enrollment. Because of the positive results obtained from the small sample size, it can be suggested that this study be replicated on a larger sample size.

Another limitation in this study was that a specific skill was not measured. In an undergraduate distance education course, monitoring the students and measuring a skill can be challenging. Students do not typically hold jobs in a food manufacturing facilities, so

monitoring them in their place of work is not possible. It is also a challenge because the student cannot be monitored in the classroom. In the future, a specific skill will be targeted, and a way to measure this skill via distance education will be explored.

2.17 Conclusions and Future Work

The multimedia case study had a positive effect on students in an introductory HACCP course. Students experienced significant improvements in knowledge, and there was a significant change in behavioral intentions ($P < 0.05$). The multimedia case study aided in the student's ability to understanding difficult information.

Studying the skills students gain through completion of the course would be another suggested course of study for the future. The development of skills is important for students to obtain, and determining what skills students should be successfully developed after course completion and the extent to which the skills are to be developed should be studied in detail.

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CHAPTER 3: Introduction

Pathogen Reduction Using Multiple Hurdle Technique and Encapsulated Nisin During Extended Storage of Shelf Stable Meals Ready to Eat (MRE)

Abstract

Meals Ready to Eat (MRE) are shelf stable meals used to provide nourishment to military personnel while in the field. To meet the growing demand to sustain these individuals while engaged, individually packed and shelf stable sandwiches have been developed. These MRE sandwiches utilize a multi-hurdle approach as well as the addition of the bacteriocin nisin to limit the growth of pathogenic bacteria, molds and yeasts. All sandwiches possess water activities between 0.88 and 0.95 and a pH of between 5.0 and 6.0. Nisin was added to the sandwiches in an encapsulated form to inhibit the growth of pathogenic microorganisms of concern over an extended timeframe by slowly releasing the antimicrobial. The objective of this study was to determine if pathogens of concern could survive or grow in a shelf stable MRE meat sandwich over the course of a year.

Three different multiple hurdle combinations of MRE sandwiches were inoculated with a cocktail in triplicate of approximately 10^6 CFU/g with three different strains of three different pathogens: *Staphylococcus aureus*, *Salmonella* spp. and *Listeria monocytogenes*, separately. These sandwiches were stored at 26.7°, 37.7° and 48.8°C and were sampled over the course of twelve months. The study indicated that the amount of pathogens present declined over time and that nisin was active through the entirety of the experiment.

3.1 Introduction

Foodborne illness is a public health concern as one in six Americans become ill with a foodborne illness every year (CDC, 2014). *Staphylococcus aureus, Salmonella spp.* and *Listeria monocyctogenes* are three important pathogens leading to foodborne illness in the United States. In order to limit the extent of foodborne illnesses, focusing on how to inhibit pathogens in specific food products is paramount particularly to those individuals engaged in military operations.

3.2 MRE Sandwiches

Meals Ready to Eat, commonly known as MREs, are shelf stable highly caloric compact meals used in the military to nourish soldiers. Feeding soldiers when they are in the field efficiently and effectively has been a concern of armies since the beginning of time (Guttman, 2010). An individual meal was developed by the Department of Defense in the 1950's, and the design evolved into a sealed plastic pouch called a Meal, Ready-to-Eat in 1975 (Guttman, 2010). This container has continued to evolve throughout the years, improving with new technology. The meals must withstand harsh temperatures and rough handling and provide nutritional requirements deemed necessary by the Office of the Surgeon General (Hawver, 2006). The MRE's are used when military personnel are in the field and not able to eat typical prepared food. MRE's contain approximately 1,200 kcals and between 1.6g – 2.3 g of sodium per meal (Feagans et al., 2010). There are at least 24 different types of MRE sandwiches for variety, and new items are made after input from personnel (Hawver, 2006).

Although MRE's are necessary for sustaining military personnel, some individuals need meals on the go. Troops on fast assault missions, specifically special operations, benefit from having meals they can consume on the go (Guttman, 2010). Combat personnel have expressed interest in the development of on-the-go meals and snack MREs, specifically sandwich-like meals (Hawver, 2006). Developing a safe sandwich in a pouch that provides nutrients to individuals unable to stop for a meal is important to military success. MRE meals are designed and developed based on an individuals evaluations and tastes, but it is important to ensure that the meal will not cause illness (Hawver, 2006).

A MRE sandwich is considered to be a ready-to-eat (RTE) product, since the food will not undergo a thermal process before consumption. The United States Department of Agriculture (USDA) defines RTE products as a meat or poultry products that can be consumed without further processing steps (USDA FSIS, 2014). Further processing steps can be added, but are not necessary for the safety of the product.

3.3 Microbiological Issues

MRE meals must meet a series of strict standards before they are to be distributed to the military. They are formulated to last 3 years at 26.7°C and 6 months at 27.7°C (Aylward and Zanchi, 2000). Sandwiches developed as on-the-go snacks must remain safe for three years. In accordance with taste, sandwiches are commonly enjoyed and meat supplies additional protein for combat personnel active in the field. In order to ensure safety, sandwiches that are to be shelf-stable for a significant amount of time must utilize a multiple-hurdle approach.

3.4 Hurdle Technology: A Multiple Hurdle Approach

Hurdle technology is described as using different types of preservation techniques to ensure target organisms of concern are not able to grow (Lester and Gorris, 1995). This technique was utilized in the early 1980's by the meat industry to inhibit bacterial growth on shelf-stable sausages (Lester and Gorris, 1995). By combining the use of different techniques, bacterial growth is inhibited because organisms have to overcome each barrier to the environment. Focusing on one technique can be problematic, because one barrier may become breeched, but multiple barriers together make it increasingly difficult (Lester and Gorris, 1995). Combining two or more hurdles to destroy bacterial cells of concern has been a common trend since the 1980's (Corbo et al., 2009). There are a variety of ways to achieve a multiple-hurdle effect including the focus on the pH, water activity, packaging and the addition of antimicrobial agents. For example, by focusing on multiple hurdles including temperatures, pH's and salt concentration, *Listeria monocytogenes* could be inhibited at great levels (Cole et al., 1990).

pH

By reducing the pH, pathogens have less of a chance of survival in the food matrix (Hobbs, 1983). Bacterial strains have a pH range that there are able to grow in, and within that range conditions that their growth is optimal (Hobbs, 1983). Typically, most bacteria grow between a pH range of 5 and 9 (Hobbs, 1983). Controlling the pH of a food matrix has positive effects on food safety, as specific pathogens can be prevented from growing and limited in survival.

Water Activity

Water activity is the ratio between water vapor pressure of the food and the vapor pressure of pure water. More simply stated, it is the amount of unbound water in a food product that can support the growth of bacteria, molds and yeasts (Fernández-Salguero et. al., 1993). The water activity of a food effects bacterial growth because these organisms need water for survival (Fernández-Salguero, 1993). The greater the water activity, the more free water is available in the food matrix for bacteria to utilize. The majority of bacteria prefer a water activity above 0.95 (Fernández-Salguero et. al., 1993) and the majority of foods have a water activity of 0.95 or higher (Food and Drug Administration, 2015). However, some pathogens grow better than others at low-water activities. For instance, *Salmonella* spp. and *Staphylococcus aureus* are both notorious for surviving and sometimes flourishing in low-moisture environments, respectively (Doyle, 2010). Most pathogenic bacteria however don't grow below 0.93. A food that is considered having a low-water activity has a water activity of 0.70 or less (Santillana et. al., 2013) while a water activity of 0.85 or lower is considered exempt from regulations regarding low-acid canned foods and acidified foods (Food and Drug Administration, 2015) due to the fact even *S. aureus* cannot produce toxin at those levels.

3.5 *Listeria monocytogenes*

Listeria monocytogenes is a ubiquitous foodborne pathogen linked to listeriosis that may cause miscarriages, severe illness, and even death (Scheff, 2003). *L. monocytogenes* is a gram positive, facultative anaerobic rod-shaped bacteria and is considered to be a psychrotroph, growing at temperatures ranging between -0.4 and 50°C (Farber and Peterkin,

1991). The known pH value for growth is between 4.3 and 9.6 and a water activity of 0.90 or greater (Harwig et al., 1991). *Listeria* is considered to be halotolerant and grows well in foods with higher salt concentrations (Juck et al., 2010). Cole et al. conducted a study comparing temperature, salt concentration and different pH concentrations determined that survival in a high-salt concentration was temperature dependent, but growth of *L. monocytogenes* was observed in salt concentration up to 12% (Cole et al., 1990).

L. monocytogenes is common in food-processing facilities because it is an environmental contaminant and it maintains the ability to form biofilms and continue replicating in these environments, surviving the use sanitation chemicals (Pan et. al, 2006). The bacterium enters the processing facility by employees, cross contamination between raw and cooked products or on contaminated ingredients. *L. monocytogenes* is commonly linked to RTE foods, since these foods do not require a thermal process before consumption (Faber and Peterkin, 1991).

L. monocytogenes is of concern in RTE meat products because of the large number of outbreaks associated with this pathogen meat related products. MRE sandwiches are typically held at room temperature but can sometimes reach temperatures as high as 37.7° C when military personnel are in areas with warmer climates. This increases the need for a multi-hurdle approach to combat pathogens in this ready-to-eat product.

3.6 *Salmonella spp.*

Salmonella spp. are members of the enterobacteriaceae family, a large family of gram-negative organisms. *Salmonella* is a rod-shaped, facultative anaerobic pathogen (Doyle,

2012) and is a leading cause of bacterial acute gastroenteritis. *Salmonella* spp. has been estimated to contribute to 80.3 million cases of gastroenteritis globally through foodborne routes each year, and is responsible for approximately 155,000 deaths annually. (Majowicz et. al., 2010). In the United States, *Salmonella* is estimated to cause 1,027,561 illnesses a year (CDC, 2014). The symptoms of *Salmonella* spp. are usually self-limiting, but can be severe and generally include diarrhea, fever, vomiting and abdominal pain (D'Aoust, 1989).

Salmonella spp. are of concern because of the ability to grow at conditions outside of its typical growth range and survive in harsh environments (Doyle, 2012). Certain *Salmonella* spp. have grown to temperatures of 54°C and as low as 4°C, even through the temperature range for *Salmonella* growth is between 25°C and 43°C (D'Aoust, 1989). The optimum pH growth range of *Salmonella* occurs between of 6.5 to 7.99, but due to the pathogens adaptability, growth between 3.99 and 9.5 has been observed (Doyle, 2012). The adaptability of this pathogen is concerning because if cells are predisposed to a condition, low pH for example, pathogen has a greater chance being able to survive being in a low pH in the future (Doyle, 2012).

Salmonella is able to grow in foods with a water activity of 0.95 or higher (Sperber, 1983). Even though *Salmonella* cannot grow in products with low water activities, there is a possibility for survival (Farakos et. al., 2014). The ability to survive harsh environments has led to many outbreaks, specifically in low-moisture environments (Doyle, 2012). *Salmonella* is the leading cause of outbreaks in foods with low water activities and many studies have been conducted to show that *Salmonella* can survive in foods for years (Farakos et. al.,

2014). Foods rich in fat content have been shown to act in a protective nature for *Salmonella*, increasing the chance of survival. For example, in a high-fat formulation (65% fat), a two log increase of *Salmonella* was present as compared to low-fat peanut butter with 19% fat (Ailves et. al., 2013).

3.7 *Staphylococcus aureus*

Staphylococcus aureus is a coccus, or round shaped, gram-positive, facultative organism (Doyle, 2012). Humans are the main source of *S. aureus* as 30-50% of humans are colonized by *S. aureus* (Scheff, 2001). The organism is found in nasal passages of humans, and is associated with human handling of a food product (Jablonski and Bohach, 2001).

S. aureus has a short incubation time and is usually self-limiting. The symptoms of *S. aureus* include vomiting, abdominal cramps, nausea, and diarrhea (Doyle, 2012). *S. aureus* affects the human population through intoxication, which is when the bacteria release exotoxins into a food product, and the now toxicogenic product, is consumed (Jablonski and Bohach, 2001).

S. aureus is considered to be one of the best non-spore forming pathogens at persisting in the environment outside of a human host and has been shown to survive in the environment for two weeks (Doyle, 2012). *S. aureus*, although a poor competitor, can produce toxin at a water activity of 0.86 (Valero et al., 2009) and grow at water activities as low as 0.83. *S. aureus* must be at a concentration of at least 10^5 in order to release the toxin to cause the infection in humans (Doyle, 2012).

3.8 Nisin

Nisin is a bacteriocin has been approved for use in food and beverages in the United States by the Food and Drug administration as a food preservative since 1988, but it was developed for commercial use in 1953 in England (Punyauppa et. al, 2015). Initially, the bacteriocin was used in cheese products and is currently being used in a variety of products.

This bacteriocin is an antimicrobial peptide that is produced by lactic acid bacteria, specifically *Lactobacillus lactis* subsp. *Lactis* (Harris et. al, 1992). Nisin is effective against many strains of gram-positive bacteria, but does not work against many gram-negative strains due to the outer membrane layer in gram-negative organisms (Punyauppa et. al, 2015). However, gram-negative organisms can be affected by nisin if their outer membrane has been weakened due to other outside factors such as osmotic shock, or disrupting the lipopolysaccharide layer in the outer membrane (Harris et. al, 1992).

Nisin destroys bacterial cells by lysing the cells and eliminating the outgrowth of spores (Harris et. al, 1992). Nisin targets the cytoplasmic membrane of vegetative cells. The catatonic portion of the cell membrane interacts with the phospholipids head allowing for the hydrophobic part of nisin interacts with the membrane core (Joo et al., 2012). Typically, bacteriocins latch onto a cell receptor, but nisin disrupts the membrane of the cell and creates an ion channel or pore in the cell due to reorganization of the phospholipid (Joo et al., 2012; Punyauppa et. al, 2015). This creates an increase in the permeability of the membrane, lysing the cell.

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CHAPTER 4: Review of Literature

Pathogen Reduction Using Multiple Hurdle Technique and Encapsulated Nisin During Extended Storage of Shelf Stable Meals Ready to Eat (MRE)

4.1 *Staphylococcus aureus*

Staphylococcus aureus is a gram-positive organism discovered in 1880's as a cause of foodborne illness associated with cheese in the United States (Bennett et al., 2013; Deurenberg and Stobberingh, 2008). *S. aureus* is, ubiquitous in the environment and is commonly found in food due to environmental, human and animal contamination (Bennett et al., 2013). Approximately 30-50% of humans carry *S. aureus* in their nasal passages, throats, hair or skin making human contamination a common source of contamination (Forsythe, 2011).

S. aureus is typically associated with meat, meat products, salads, dairy, and cream-filled bakery products. Once in the food, *S. aureus* produces a heat-stable enterotoxin at levels of 10^5 or greater (Bennett et al., 2013). *S. aureus* can grow at a range of between 7-48°C, a pH between 4-10 and a 0.83-0.99 water activity. However, the toxin is only produced in a range of 10-48°C, at 4.5-9.6 pH and a 0.87-0.99 water activity (Forsythe, 2011).

S. aureus causes an infection usually two to six hours after ingestion of the enterotoxin. On average, 1.0ug of toxin is needed to produce symptoms, but in immunocompromised persons, only 100-200 ng is needed (Bennett et al., 2013). The symptoms caused by Staphylococcal infection include nausea, vomiting, and, abdominal cramping but are typically self-limiting (Forsythe, 2011).

4.2 *Listeria monocytogenes*

Listeria monocytogenes, a gram-positive, bacillus-shaped organism, is a major pathogen of concern in raw meat and poultry products around the world and is a causative leader of death amongst foodborne pathogens (Donnelly, 2001; Kara et al., 2014). Listeria has been implicated in millions of pounds of recalls annually resulting in a public health concern and an economic burden (Nguyen, 2008).

L. monocytogenes was first described in the 1920's for its infectivity of small animals including rodents, but it was not until 1936 that it was described as infectious in humans (Crum, 2002). *Listeria* is a zoonotic pathogen, ubiquitous in the environment and found commonly in the soil, plant material, water, and feces of animals including but not limited to: cattle, sheep and goats (Donnelly, 2001).

L. monocytogenes has the capability of growing in a wide range of pH (4.3-9.6), temperatures (-1.5-50˚C) and has been shown to survive in sodium chloride concentrations of up to 12% (Cole et al., 1990; Donnelly, 2001). *L. monocytogenes* is facultative anaerobic organism that is nonsporeforming consisting of 13 serotypes, of those, only three account for greater than 90% of human illness (Dussurget et al., 2004). These strains include 4b, 1/2 a and 1/2 b, but the type of infection demonstrated by the host is not linked to a specific serotype (Crum, 2002). *L. monocytogenes* works by invading the epithelial cells and replicates in the host cells cytoplasm. From there, *L. monocytogenes* enters neighboring cells by making a secondary vacuole into other cells by using a double membrane protrusion.

L. monocytogenes is a systemic disease, infecting both humans and animals

intracellularly (Hamon et al., 2006). The infective dose of *L. monocytogenes* is dependent on the individual consuming the organism, but it has been estimated that fewer that 1,000 total organisms can cause illness CFU/ml (Chen, 2014). *Listeria* is most commonly associated with raw vegetables, unpasteurized milk, poultry, deli meats, soft cheeses, and other food items (Crum, 2002). *L. monocytogenes* causes gastroenteritis, meningitis, encephalitis, abortions and perinatal infections, and listeriosis, which has a 30% mortality rate (Dussurget et al., 2004). Due to the high mortality rate associated with *L. monocytogenes* and the fact it is cause for concern in RTE product, total absence of *L. monocytogenes* in every 25 g or mL food sample is required amongst food processors (Kara et al., 2014).

4.3 *Salmonella enterica*

Salmonella is a bacillus-shaped, gram-negative, facultative anaerobe, nonsporeforming microorganism and a member of the enterobacterioaceae family (Forsythe, 2011). *Salmonella* is of global health concern and is spread through many different routes, but is strongly associated with foodborne illness (Schatten, 2007).

Salmonella has an optimum growth temperature of 38°C with a minimum growth temperature around 5°C. *Salmonella* also has many seravars, also known as serotypes, consisting of over 2324 different varieties (Schatten, 2007). *Salmonella* infects its host by entering the gut and invading the epithelium of the small intestine. Once there, *Salmonella* multiplies and causes an inflammatory response to occur (Forsythe, 2011).

There is a wide range of foods typically associated with *Salmonella* including, but not limited to, raw meats, poultry, eggs, milk and dairy products, fish, shrimp, frog legs, yeast,

coconut, sauces, salad dressing, cake mixes, cream-filled desserts, dried gelatin, peanut butter, cocoa, and chocolate (Forsythe, 2011). Contamination can occur through poor temperature control, poor handling practices, and cross contamination of raw and processed foods (Schatten, 2007).

The symptoms of *Salmonella* include diarrhea, nausea, abdominal pain, mild fever and chills, and sometimes vomiting and headache. Symptoms occur between 16 and 72 hours, lasting between 2-7 days, typically being self-limiting, but can cause death, specifically in the immunocompromised populations (Anonymous, 1997).

4.4 Nisin

Nisin is an antimicrobial agent that inhibits a series of microorganisms specifically certain strains of gram-positive microorganisms. Nisin is produced by *Lactobacillus spp.*, naturally occurring in raw milk (Delves-Broughton, 1990). Nisin is effective in inhibiting spores of microorganisms, but is more effective at inhibiting some spores than others (Delves-Broughton, 1990).

Nisin destroys bacterial cells by causing leakage from the cytoplasm or in some cases lysing the cells (Harris et. al, 1992). Nisin targets the cytoplasmic membrane of vegetative cells. The catatonic portion of the cell membrane interacts with the phospholipids head allowing the hydrophobic part of nisin to interact with the membrane core (Joo et al., 2012). Typically, bacteriocins latch onto a cell receptor, but nisin disrupts the membrane of the cell and creates an ion channel or pore in the cell due to reorganization of the phospholipid (Joo et al., 2012; Punyauppa et. al, 2015). This creates an increase in the permeability of the membrane, lysing the cell.

Nisin also has the ability to eliminate the outgrowth of spores (Harris et. al, 1992) and this action is considered sporicidal (Hitchens et al., 1963). Nisin targets the ability of the spore to coat rupture, which occurs during the spore swelling stage of germination (Delves-Broughton, 1990).

The antimicrobial efficacy of nisin is dependent on many factors (Millette et al. 2007) including dose, food matrix interactions and specific food chemistry. Even though nisin has been shown to work in vitro, when applying nisin directly to food products, many different problems can occur. The inhibitory effect of nisin to is dependent on the spore load, temperature of heat shock, length of heat shock and pH (Scott and Taylor, 1981).

Scott and Taylor (1981) compared different variables that influence the effectiveness of nisin in the food matrix. Stock solutions of nisin containing between 0-2,000 IUs were developed and different combinations of pH, time and temperature and spore load were explored. The study suggested that nisin is more effective in a more acidic pH of 6 as compared to 7 or 8, that nisin is more effective on a lower spore load, and that nisin is more effective on heat-damaged cells. It was also suggested that the amount of nisin available to inhibit microorganisms of concern was diminished due to the increased length of heat shock, as well as the increased temperature of the heat shock. By keeping the pH of the food matrix low and ensuring that the heat applied to the food product, at approximately 80°C for *C. botulinum* spores, the effectiveness of nisin will improve. Based on these results, it can be determined that the targeted food matrix plays a significant role in the efficacy of nisin's inhibitory effects suggesting that the food matrix that nisin is inserted to must be taken into account.

Pathogens such as *Clostridium botulinum*, *Staphylococcus aureus*, *Bacillus cereus* and *Listeria monocytogenes*, as well as spoilage microorganisms including *Lactobacillus* spp. have been shown to be inhibited by nisin in vitro, but they have not been effectively inhibited by nisin in actual meat products, specifically ground beef products (Cutter and Siragusa, 1998). Fresh meat contains enzymatic components that are suspected to inactivate nisin (Rose et al., 1999). Although there are complications, nisin has been used as successful meat preservative (Siragusa et al., 1999). The addition of nisin has also not been shown to have any antagonistic effects on other aspects of a multi-hurdle approach (ex. water activity, pH) while in low temperatures in meat products (Hampikyan and Ugur, 2009).

Rose et al. (1999) compared different meat products to determine the amount of residual nisin. A solution of 50, 125 and 250 ug of nisin in 1mL 0.02N HCL was added to fresh meat, cooked meat, fresh juice, and cooked meat juice. Cooked-meat and cooked-juice samples were heated in a boiling water bath for five minutes and then cooled to 4°C. Meat samples were soaked in 1 mL of solution for one hour at 4°C and juice samples were stomached for one minute. The meat samples were then vacuum packed and stored overnight to await assays to determine nisin activity. Rose et al. (1999) discovered that cooked meat retained nisin activity while raw meat did not, indicating that fresh meat contains a component, specifically an enzyme due to the nonoccurrence in cooked meat, which reduces nisin's activity. This indicates that nisin should be added to cooked meat or in a vesicle in which the enzyme will not affect the activity of nisin.

Cutter and Siragusa (1998) combined nisin with a meat-binding system to apply it to fresh and vacuum packaged meat surfaces to inhibit *Brochothrix thermosphacta,* a spoilage microorganism. *B. thermosphacta* was sprayed onto the meat system at levels of 10^3-10^4 CFU/g. A nisin concentration of 10 ug/ml combined with Fibramix, the meat-binding system, was evenly sprayed on the meat tissue which was refrigerated for up to two weeks. Nisin was still effective in the meat-binding system after the 14 days, and it was suggested that adding nisin to the meat-binding agents would be a beneficial delivery mechanism for nisin to a meat system.

Instead of using a meat-binding agent to deliver nisin to a meat system, Siragusa et al. (1999) used a polyethylene base plastic film with 0.1% nisin and 0.05% nisin by weight to determine the retention of nisin. *B. thermosphacta*, a spoilage microorganism that grows optimally at refrigerated temperatures, levels of 1.0×10^6 CFU/ml were inoculated into beef carcass surface tissue sections. After 20 days, there was significantly less ($\log_{10}$ 5.8 vs 7.2 CFU/cm^2) *B. thermosphacta* in the samples with nisin as compared to the ones without. This study highlights another delivery method for nisin to encourage an antimicrobial effect to enhance food safety and decrease food spoilage.

Nisin and *Salmonella*

Nisin is known as a broad-spectrum bacteriocin against gram-positive strains of bacteria. *Salmonella*, being a gram-negative strain, has been shown to not be inhibited by nisin (Chung et al., 1989; Cutter and Siragusa, 1996). However, other studies have shown that *Salmonella* has been inhibited by the properties of nisin (Stevens et al., 1991).

Gram-negative organisms are typically resistant to nisin because of their outer membrane. The outer membrane protects gram-negative organisms by preventing unwanted substances from entering the internal cellular environment (Nikaido, 2009). The outer membrane consists of a large bilayer of lipopolysaccharide in the outer leaflet and phospholipids on the inner leaflet (Tokuda, 2009). These two layers create a selectively permeable layer for the cell. In the outer membrane, the rate at which substances are able to permeate into the cell is approximately two orders of magnitude slower than without the outer membrane (Nikaido, 2009). However, when the outer membrane is weakened or altered by osmotic shock or chelating agents for example (Harris et al, 1992) gram-negative bacterium may become more susceptible to nisin.

In 1991, a study conducted by Stevens et al. aimed to evaluate the effectiveness of nisin when combined with a chelating agent against *Salmonella* determined this combination was successful in reducing different strains of *Salmonella* in vitro. A chelating agent, EDTA, was combined with 50 ug of Nisin and exposed to the pathogen for one hour at 37°C. There was a 3.2-6.9 log reduction of the *Salmonella spp*. when nisin and EDTA were exposed, but no significant reduction in pathogen when either of the agents worked alone. A chelating agent weakens the outer membrane layer of the gram-negative organisms by binding magnesium ions to the outer membrane layer, disrupting the outer membrane layer and producing cells that are more susceptible to antimicrobial agents (Prudêncio et al., 2014; Stevens et al., 1991).

Applying nisin as the only inhibiting agent to meat products was not shown to be effective against gram-negative organisms, specifically *S. typhimirium* as well as *S. aureus* and *L. monocytogenes* (Chung et al., 1989). Chung et al. soaked lean ground beef for ten minutes at room temperature with nisin (10^4 IU/g) then inoculated the samples with 10^7 CFU of *Salmonella typhimirium* ATTC 14028. Chung postulated that nisin did not have a significant effect on the bacteria binding to the meat because a chelating agent was not added (Chung et al., 1989). Another study, however, was conducted with *Salmonella* in lean beef products using a combination of nisin and a chelating agent that did not indicate inhibition of the organism suggesting that the activity of the nisin-chelating agent complex may not be effective in certain food matrices (Cutter and Siragusa, 1996). Cutter and Siragusa (1996) inoculated lean ground beef with 10^9 CFU *S. typhimirium* ATTC 14028. EDTA, citric acid monohydrate, DL-lactate and sodium hexametaphosphate was combined with 50 ug/ml of nisin to make a final nisin concentration of 1% weight/vol. The meat was held at 5°C for three days and no significant difference was observed between the strains.

Prudêncio et al., 2014 analyzed the use of nisin against *Salmonella typhimirium* under various environmental conditions. A chelating agent, EDTA, was used in combination with nisin and cell viability was determined after 48 hours in vitro. The 10^5 CFU/ml *Salmonella* ATTC 14028, 200 AU/ml of nisin and EDTA mixture were subjected to pH levels of 5.0, 5.3, 5.9, 6.1, 6.9, and 7.1, at temperatures of 10, 15.1, 27.5, 39.9 and 40. Prudêncio et al., 2014 determined that nisin was more effective against *S. typhimirium* in a pH close to neutral. Although, nisin has been shown to work over a range of temperatures and be more

stable at a lower pH (Scott and Taylor, 1981) the chelating agent EDTA is more effective at a neutral pH, suggesting that the chelating agent plays a very important role in inhibiting *S. typhimirium*.

Nisin and *Listeria*

Listeria monocytogenes has been shown to be inhibited by nisin in a variety of food products (Nguyen et al., 2008) including raw ham products, Turkish sausages, ground beef, frankfurters, as well as others (Boualem et al., 2013; Nguyen, 2008; Hampikyan and Ugur, 2007; Morioka et al., 2013).

Boualem et al. (2013) conducted a study encapsulating nisin in raw meat products to improve its effectiveness. Nisin was encapsulated in Dipalmitoylphosphatidylcholine (DPPC), a liposome and was tested in the raw and encapsulated form in raw and cooked ground beef. Nisin (3200 AU/ml) was mixed with the meat then the raw meat was held at 4°C for up to 24 hours to allow for the nisin to react while the cooked meat was cooked to an internal temperature of 80°C, cooled and tested immediately. Nisin was detected as active in both the free and encapsulated form, but only in the encapsulated form for the raw meat revealing that encapsulating nisin ensures that the antimicrobial will remain active in the food system and prove that nisin can inhibit *L. monocytogenes*.

In a proof of concept study conducted by Nguyen et al. (2008), a bacterially produced cellulose film was developed and contained nisin to inhibit 10^6 CFU/ml *L. monocytogenes* in vacuum-packaged frankfurters. Different concentrations of nisin were on the films ranging from 156 IU/ml$^-$ to 10,000 IU/ml$^-$and were exposed to the product for six

hours. Films produced with an IU amount of 2500/ml or higher had a significantly reduced amount of *L. monocytogenes* by approximately two logs after 14 days of storage.

Chicken burgers formulated with 25 ug/g, 50 ug/g, and 100 ug/g IU's of nisin were inoculated with a single-strain cocktail of *L. monocytogenes* ATCC 7644 at a level of 10^4 and 10^6 and sampled over a 25-day period. Nisin was shown to reduce the amount of *L. monocytogenes* by approximately two logs throughout the 25-day period. Sandwiches with higher concentrations of nisin proved to have a greater inhibitory effect on *L. monocytogenes* (Kara et al., 2014). The inhibition effect of *L. monocytogenes* was also shown to be dependent on the amount of nisin present in the sandwich, finding that a greater concentration of nisin is needed in order to get more than two logs of inhibition (Kara et al., 2014).

Adding the antimicrobial nisin to a meat product has been shown to inhibit *L. monocytogenes* at certain concentrates, but Morioka et al. (2013) found that the water activity of the raw meat also plays an important role in the inhibitory effect. *L. monocytogenes* serotype Scott A was inoculated into raw ham in vitro. It was found that *L. monocytogenes* did not grow in the raw meat in a water activity under 0.93, but when nisin was added at 12.5 mg/kg, *L. monocytogenes* was not able to survive in a water activity of 0.94. This demonstrates that a multiple-hurdle approach to limiting the growth of *L. monocytogenes* is beneficial.

Different concentrations of nisin and their effects on *L. monocytogenes* were explored in Turkish-fermented sausages by Hampikyan and Ugur (2009). This dish is made by mixing

sheep, beef or water buffalo meat with fat, garlic, nitrite, sugar, salt and other spices. Six treatments were conducted, adding 10^6 CFU/g *L. monocytogenes* with the dough and then were treated with 5ug, 10ug, 25ug, 50ug, 100ug of nisin and a control. Analyses were conducted to up to 30 days and it was found that concentrations of nisin with 50 ug/g and 100 ug/g at days 20 and 25 resulted in no *L. monocytogenes* cells. It was suggested that the greater the concentration of nisin, the greater inhibition of *L. monocytogenes*. At a concentration of at least 50 ug/g, the ability to detect *L. monocytogenes* diminished in Turkish sausages, suggesting that this concentration of nisin will be effective in similar meat products.

Nisin and *Staphylococcus aureus*

Nisin has been proven to inhibit *Staphylococcus* in a series of in vitro based studies and in vivo studies, specifically in food products containing meat (Chung et al., 1989). Although nisin inhibits this organism, there are limitations in formulating nisin in meat products (Millette et al., 2007).

Fresh lean beef was inoculated with 10^7 CFU of *S. aureus* ATCC 25923 and then nisin (10^4 IU/ml) to show an inhibitory effect. *S. aureus* was inhibited at 5°C but in ambient temperatures, the inhibitory effects only lasted for three days (Chung et al., 1989). This study indicated that nisin does not function well when incubated at low temperatures, and other approaches must be implemented in addition to nisin formulation, such as water activity or pH control.

Millette et al. (2007) also determined that *S. aureus* ATTC 29212 was inhibited by nisin in ground beef. In this study, round beef steak was inoculated with 10^4 CFU/cm^2 of *S. aureus* and stored at 4°C for 14 days. Levels of 500 and 1000 IU/ml of nisin were coated in an alginate film to stop the nisin from bonding to the meat product directly at 4°C. The nisin films were formed into beads and added to the meat product directly (Millette et al., 2007). The product was stored at 4°C and sampled at five and seven days. There was a statistically significant reduction of *S. aureus* observed over time (Millette et al., 2007). This study revealed that nisin exhibited inhibitory effects on *S. aureus* while impregnated in a film suggesting that *S. aureus* can be inhibited by nisin in vivo.

4.5 Encapsulated Nisin

Using nisin in meats at ambient temperature has been shown to have no effect over inhibition of microorganisms over time as nisin degrades, which causes there to be less of an inhibitory effect against microorganisms (Chung, 1989). In order to combat this, nisin may be encapsulated and has been shown to increase activity for longer periods of time (Boualem, 2013).

As aforementioned, there are difficulties when adding nisin directly to a meat products, unless the nisin is added at high concentrations (Boualem, 2013); however, all of the nisin is not able to be absorbed into the food system. Encapsulating the nisin allows the preservative to be released over time, protects the substance from being disrupted by the food matrix and extends the period of time the antimicrobial is effective. (Boualem, 2013; Huq, 2014). In a study that examined various concentrations (16, 31 and 62 ug/ml) of encapsulated nisin and free nisin in raw ham at 4°C for 28 days, there was no more active nisin available

in the free nisin after 28 days, but there was available nisin in encapsulated form. The encapsulated nisin significantly reduced *L. monocytogenes* more than unencapsulated nisin between 1.5 and 3.03 logs dependent on concentration (Huq, 2014).

The encapsulation process has been described as incorporating food particles, cells, materials or other substances into small capsules (Gibbs et al., 1999). Nisin has been encapsulated in a variety of matrices. Boualem et al. (2013) used a liposome structure to encapsulate the nisin. The structure was formed when polar lipids were dissolved in aqueous solutions (Mertins, 2005). The encapsulated liposome structure works as the single or multiple bilayer or polar lipids encasing the nisin that will melt away when added to heat allowing the nisin to be released over time as the liposome structure gets destroyed (Boualem et al., 2013). Dipalmitoylphosphatidylcholine (DPPC) and lecithine were the two phospholipids explored for the creation of liposome formation. DPPC was shown to be of better use for meat products that were cooked post-processing as the release temperature for nisin was greater, 45°C for DPPC as compared to 25°C for the lecithine (Boualem et al., 2013).

Alginate is another common substance used to encapsulate nisin (Millette et al., 2007). The nisin covalently links to the alginate substance, preventing the nisin from being broken down and remaining active (Millette et al., 2007). Alignate beads can then be stored for long periods of time, keeping the nisin intact. By using an encapsulation process, pathogens inhibited by nisin in vitro can be successfully reduced in meat products (Boualem et al., 2013; Millette et al., 2007).

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CHAPTER 5: Material and Methods

5.1 Bacterial Assay Materials and Methods

Three types of MRE (meal ready to eat) sandwiches were selected for a shelf-life study occurring over the course of twelve months. The sandwiches consisted of a beef BBQ sandwich with a water activity of 0.95 and pH of 6.0, an Italian sandwich with a water activity of 0.90 and pH of 5.5, and a second Italian sandwich with a water activity with a water activity of 0.88 and pH of 5.0. The pathogens used to challenge these multiple-hurdle combinations were *Listeria monocytogenes*, *Salmonella spp.*, and *Staphylococcus aureus*.

5.2 Pathogen Strains and Growth Conditions

A cocktail of three strains of each of the following three bacteria were used for this experiment: *Staphylococcus aureus, Listeria monocytogenes,* and *Salmonella spp.*. The three strains of *L. monocytogenes* were Scott A., JO161, and LW-A46. Two of the three stains have been the cause of major outbreaks, and all three strains have been used in prior published studies, reducing the probability of stain interference (Cheng, 2014). Each strain was streaked onto the selective agar Modified Oxford Media, (MOX) (Oxoid CM0856, Listeria Selective Agar Base, United Kingdom) and placed into an incubator of 37°C to ensure purity.

Heidelberg, Typhimrium and Enteritidis strains of *Salmonella* were used in the experiment by first streaking each strain onto XLD media (Difco, XLD Agar, Sparks, MD, USA) to ensure purity. The three strains used are associated with low-water activity outbreaks and because the sandwiches of concern contain a lower water activity, strains that

have been shown to survive in low-water activities were used (Grasso et al., 2014). New England, Wisconsin and ATCC strain number 25923 were the strains used to represent *S. aureus* by first streaking onto Baird Parker Agar (Difco, Baird Parker Agar Base, Sparks, MD, USA) to ensure purity.

Once each strain of each bacteria had been streaked onto the selective agar and placed in the incubator at 35°C for 48 hours, the plates were removed. Each strain of the three bacteria was then grown separately at 35°C for 24 hours +/- 2 hours by transferring a single typical colony into 9 mL of Trypticase Soy Broth (BBL, Trypticase Soy Broth, Sparks, MD, USA) to achieve approximately 10^8 CFU/ml (Andrews et al., 2014; Bennett and Lancette, 2001; Hitchins and Jinneman, 2011). The individual colonies were chosen based on visual inspection of the colonies. These pathogens were confirmed by growth on selective agar, and the *Listeria monocytogenes* was confirmed by PCR and rapid listeria test kits (Thermo Scientific Remel Micro ID, Listeria Identification System). On XLD agar, *Salmonella* was red to yellow with a black center while *L. monocytogenes* appeared as small colonies with dark halos surrounding them. *S. aureus* was plated on Baird Parker agar and a typical colony were shiny black colonies approximately 1.5 mm surrounded by a zone of clearing (Bennett and Lancette, 2001; Hitchins and Jinneman, 2011).

For each of the three pathogens aforementioned, 1 ml of each strain was then transferred into a centrifuge tube. Next, the pathogens were spun down into a pellet using a centrifuge at 1200 rpm for 5 minutes (Eppendorf Centrifuge 5145), the supernatant was removed and then the cells were re-suspended in 1 ml of buffered peptone water. This was done to ensure that the broth the cells were stored in would not influence in the experiment.

The three strains of each bacterium were then combined into 3 ml to create a homogenous mixture, which was used for inoculation immediately after preparation. Each three-strain cocktail was inoculated into separate sandwiches to ensure that the different pathogens did not exhibit any antagonistic effects.

5.3 Preparation of Samples

One thousand 120 g sandwiches were formulated and encapsulated in wax microspheres with nisin at target level of 250 ppm, handmade and packed by RDI Foods. In brown pouches that were comprised of three layers of various materials including 0.002-inch thick polyethylene film, 0.00035-inch thick aluminum foil and 0.0005-inch thick polyester. This package was then laminated with polyester to serve as an outer covering (Personal Communication, 2014).

Once received, the water activities of the sandwiches were corroborated using an Aqualab water activity meter and the mixing technique for the sandwiches was determined. The inoculation process began approximately four weeks after packaging. The sandwiches were opened by using scissors flamed with a 70% ethanol solution in a biohazard hood and were gently hand mashed together using sterile gloves to ensure the water activity would remain consistent and create a homogenous environment for inoculation. The sandwiches were then weighed into allotments of 20.00 g +/- .02 using an autoclaved foil weigh dish, and deposited into a 24-ounce filter Whirlpak bag (Fisher Scientific, Nasco, 9 in x 6 in). Once inside the Whirlpak bag, 20 μL of a three-strain cocktail of one of the three bacterial pathogens aforementioned was pipetted into the sandwich. The sandwiches were then

massaged to distribute the pathogen throughout the sandwich uniformly. The Whirlpak bag was then rolled up and deposited into the foil-lined pouches in which the sandwiches were originally received. A Freshpax bag (Multisorb Technologies, Buffalo, NY, USA) was added to the each sandwich bag to scrub the existing oxygen once the pouch was sealed. The bag was heat sealed twice using a Koch Ultravac vacuum package machine (Model UV225, Item No 903225-070, Koch Equipment, Kansas City, MO) to obtain a seal but not create a vacuum on the product.

5.4 Storage of Samples

After inoculation, the sandwiches were stored in three separate incubators all maintaining a different internal temperature. A third of the sandwiches was stored at 48.8°C for one month and then reduced to a temperature of 37.7°C for the remainder of the year (Revco RTC30n, New Columbia, PA, USA). Another third of the sandwiches was stored at 37.7°C (Barnstead Labline, 403, Malaysia), while the last third of sandwiches was stored at 26.7°C (Spectrofuge 11-679-25c) (Table 1). The temperatures were chosen by the military to test a temperature of 48.8°C to mimic the hot environments soldiers have experienced while in the Middle East. The temperature of 37.7˚C was chosen because it is a common incubation temperature used to grow the pathogens of concern. A temperature of 26.7°C was chosen being reflective of normal ambient temperatures.

5.5 Sampling of Samples

The sandwiches were sampled at 0, 28, 56, 128, 156 and 364 days. At each point, sandwiches from all three temperatures and of each pathogen were sampled. The pouches were removed from the incubator and opened using scissors sterilized with 70% ethanol.

The whirlpak bag inside the preformed brown package was removed using tongs sterilized with 70% ethanol and 180 ml of sterile buffered peptone water (Difco, Sparks, MD, USA) was added. The whirlpak bag was stomached (Seward Stomacher 400 Circulator, Davie, FL, USA) for 120 seconds at 260 RPM. The sample was serially diluted using buffered peptone water, as necessary and plated in duplicate. Sandwiches inoculated with *L. monocytogenes* were plated on Modified Oxford (MOX) agar while sandwiches inoculated with *S. aureus* were plated on Baird Parker Agar as well as selective petrifilm agar (3M Petrifilm, Staph Express, St. Paul, N, USA). Sandwiches inoculated with *Salmonella* spp. were plated on XLD for identification. The plates were stored in an incubator (Barnstead Labline, 403, Malaysia) at 35°C +/- 2° for 48 hours (Andrews et al., 2014; Bennett and Lancette, 2001 Hitchins and Jinneman, 2011). Colonies were counted at 24 hours and again at 48 hours. Sandwiches that had not been inoculated with a pathogen were also sampled on all three types of agar at each sampling time and were stored and counted alongside the other samples. The entire experiment was repeated in triplicate.

5.6 Nisin Assay

The protocol for nisin assay was modified from Wolfe and Gibbons, 1996, and was the protocol that was provided to NC State by RDI for use as the standard protocol as described below.

5.7 Standards Development

Standards were made using pure nisin as nisaplin was difficult to acquire and is converted to nisin (Pongtharangkul, and Demirci, 2007). One ml of sterile 0.02 mol HCl

was added to 49 ml of sterile water and mixed followed by pH confirmation between 1.5 and 2. Three ml of Trypticase Soy broth (BBL 211768) was added to 27 ml of the HCl and water mixture (9:1 mixture). Then 10 mg of nisin (MP Biomedicals 155839) was added to 10 ml of the 9:1 mixture. This solution was centrifuged and added to each sterile 15 ml glass centrifuge tube to create the standards.

To make the standard solutions, the 500 IU standard was made first. Ten ml of pure nisin was added to 10 mL of the 9:1 mixture. To make the 400 IU standard, eight ml of the 500 IU standard was added to 2 ml of the 9:1 solution. Six ml of the 400 IU standard was added to two ml of the 9:1 mixture to make the 300 IU standard. The 200 IU standard was made by combining 4 ml of 300 standard was added to 2ml of 9:1 and the 100 IU standard was made by adding 2.5 ml of both 8:1 and 200 IU standard. The 75 standard was made by adding 3 mL of 100 and 1 mL of 9:1 and the 50 IU standard was made by adding 2 mL of 75 and 2 ml of 9:1. Adding 2 ml of 50 and 2 mL of 9:1 made the 25 IU standard. The 10 IU standard was made by adding 1 ml of 25 and 1.5 ml of 9:1, and the 5 IU standard was made with 1 mL of 10 and 1 mL of the 9:1 mixture. Once developed, the standards were stored at 4°C until used.

5.8 Sample Preparation

The sandwiches had to be liquefied in order to pipet them into the well plate. This was accomplished by adding 30 ml of peptone water to 20 grams of sandwich inside a filter bag. Once in the filter bag, the sandwich was homogenized for 120 seconds at 260 rpm using a stomacher (Seward Stomacher 400 Circulator, Davie, FL, USA). The filter bag was opened, and 10 ml of liquid, using a 10 ml sterile pipette (Fisherbrand 13-678-11E) was moved from

the bag to a disposable 50 ml centrifuge tube (VWR 89039-658). The centrifuge tubes were refrigerated with the standards until being added to the wells.

5.9 Agar and Procedure

Two containers of 250 ml of Trypticase Soy Agar (BBL 211043) and one container of 250 ml of MRS (Difco 288130) were autoclaved then air cooled to approximately 45°C. Next, a bacterial strain was added to a container. The containers were each inoculated with 2.5 ml of either *S. aureus, Micrococcus luteus* or *Lactobacillus brevis*. Approximately 30 ml of the inoculated medium was then poured into individual square plates (100mm x 100mm x 15mm). These plates were incubated at a refrigerated temperature of approximately 4°C for at least three hours to allow for solidification.

Once the inoculated plates had been incubated for the appropriate time, they were removed from refrigeration and 8mm wells were made. These wells (6-7 per plate) were made by applying suction to sterile disposable pipette tips (Fisherbrand 200 µL). Placing a finger on the opposite end of the pipette tip created the suction. After the wells were made, the standards solutions (0-500 IU/ml) and the samples were removed from the refrigerator and added to the wells (100 µL). The plates were then stored at 30°C for four days to insure growth had occurred. The zones of clearing around the wells created on each plate was measured with calipers and rounded to the nearest 1mm. This entire experiment was repeated in triplicate.

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CHAPTER 6: Results and Discussion

6.1 Pathogen Control

Listeria monocytogenes, *Staphylococcus aureus* and *Salmonella enterica* experienced a significant decline throughout the one-year study at 26.7, 37.7 and 48.8 ˚C (Figure 9-17). More specifically, in the first 28 days of the experiment, *Salmonella* and *Listeria* strains experienced a 3-log reduction from levels ranging between 10^6-10^7 and dropping to levels of 10^5-10^2 in both of the formulations of Italian sandwiches. *Staphylococcus* strains had greater survivability than *Salmonella* and *Listeria* strains, dropping to levels below 10^5 after day 58 in the majority of sandwiches, surviving at levels below 10^5 through remainder of the experiment. Nisin was active through all time points in the experiment, exhibiting antimicrobial effects for the entire 365 days.

Salmonella was the first pathogen to become non-detectable after 58 days and remained undetectable until the conclusion of the study at day 365. This finding is contradictory with a study conducted by Cutter and Siragusa (1996) in which lean beef products containing nisin and a chelating agent were inoculated with 10^9 CFU of *Salmonella*. This study revealed that *Salmonella* was not inhibited by nisin after three days. Additionally, in a study conducted by Chueng et al (1989) *Salmonella* was also not inhibited by nisin, however, multiple hurdles were not used to prevent the pathogen from growth. Lean ground beef was soaked in 10^7 of *Salmonella* and then dipped with 10^4 IU of nisin to determine if the attachment of gram negative and gram positive organisms would have different levels of attachment. It was determined that meat with nisin did not have significantly less bacterial attachment of *Salmonella*.

In both contradictory studies, however, nisin was applied directly to meat products instead of being encapsulated prior to meat exposure. In this study it was indicated that *Salmonella* was inhibited by multiple hurdles in combination with encapsulation of nisin in all three meat sandwiches and temperature combinations after day 56. *Salmonella* was present in each type of sandwich at 26.7°C after 28 days (BBQ, 3.40 log/CFU; Italian a_W 0.92, 3.92 log/CFU; Italian a_W 0.90, 1.48 log/CFU). At day 56, *Salmonella* was only detectable in the Italian 0.92 sandwich at temperatures of 26.7°C and 37.7°C. After day 56, *Salmonella* was no longer detectable in any sandwich held at any temperature.

Although *Listeria* had a two to three-log reduction within the first 28 days study in all of the sandwich types and in all temperatures positive samples occurred through day 84 (Figure 9-17). Any sample that tested positive after day 84 was verified with the rapid listeria test kit (Thermo Scientific Remel Micro ID, Listeria Identification System) to confirm presumptive positive colonies. In agreeance with Chung et al. (1989), nisin had an effect on *L. monocytogenes*, which concluded that the effects of nisin diminished throughout the four-week incubation period at ambient temperatures. In that study, fresh lean meat with 10^4 nisin was inoculated with 10^7 CFU *L. monocytogenes* and *S. aureus*, incubated at 5°C and at ambient temperatures for a total of four weeks and compared to a control meat sample. *L. monocytogenes* was significantly different from the control study, but the effects of nisin wore off over time, concluding that nisin could not bind to fresh meat (Chung et al. 1989). Both of these studies however, did not focus on encapsulating nisin particles. Contrarily, the current research focused on encapsulating nisin to facilitate binding with meat to ensure slow

release over time in combination with water activity control, to inhibit *Listeria* growth at days 156 and day 365 and maintaining levels at 10^2 or below (Figure 9-17).

In a study conducted by Boualem et al. (2013), nisin was encapsulated and mixed with raw meat and held for 24 hours at 4°C. It was determined that the nisin remained active in both raw and cooked meat. This concurs with the current research that was conducted, more specifically the encapsulation kept nisin active, thus inhibiting *L. monocytogenes* for a longer duration. Additionally, Nguyen (2008) added cellulose films containing different concentrations of nisin to vacuum packaged frankfurters and observed reduction of *L. monocytogenes* in frankfurters that had nisin films containing 2500 IU/mL or more. This is more nisin than found necessary to inhibit *L. monocytogenes*. In a study using Turkish fermented sausages different concentrations of nisin were added to determine if 10^6 CFU of *L. monocytogenes* would be inhibited (Hampikyan and Ugur, 2007). Another study suggested that levels of 50 ug (200 IU/g, Davidson et al., 2005) or greater successfully inhibits *L. monocytogenes* to undetectable levels. This agrees with the current research that suggests that levels ranging between 10 IU/mL and 500 IU/mL in combination with other hurdles inhibited *L. monocytogenes*.

This also agrees with Morioka et al. (2013) that determined a low water activity in combination with nisin aided in the inhibition of *L. monocytogenes*. Raw ham was inoculated with *L. monocytogenes*. It was concluded that a water activity of 0.93 did not allow the growth *L. monocytogenes*, but when nisin was added, inhibition occurred at a water activity of 0.94. This suggests that adding nisin to a food product will allow for formulation of products with a higher water activity while simultaneously inhibiting pathogens of concern.

Staphylococcus aureus, although a gram negative microorganism, exhibited greater survivability than both strains of *Salmonella* and *Listeria* especially in the Italian sandwiches. In the Italian sandwich with a 0.92 water activity stored at 26.7°C averaged 2.58 log/CFU on day 356 while *Listeria* and *Salmonella* were undetectable. In the Italian sandwich with a water activity of 0.90 at the 26.7°C storage temperature, *S. aureus* survived at levels of 3.26 log/CFU. Although this pathogen was present, growth levels of greater than 10^5 must be present for the toxin to be produced and illness to occur (Le Loir, 2003). After day 56 until the conclusion of the study at day 365, *S. aureus* remained below 10^5. This finding is in partial agreeance with Chueng et al (1989), where *S. aureus* was inhibited by the presence of nisin for at least one day. As mentioned above, the Chung et al. (1989) study inoculated lean ground beef with 10^7 CFU *S. aureus* and 10^4 IU nisin. Nisin inhibited the growth of *S. aureus*, but nisin activity was severely diminished over time at ambient temperatures. This study agrees that nisin can inhibit *S. aureus*, but because the nisin was not encapsulated unlike the study at hand, nisin activity did not stay active.

In another study levels 500 IU/ml and 1,000 IU/ml of nisin were coated in alginate film and added to ground beef steak. After five and seven days at 4°C, levels of *S. aureus* were significantly reduced (Millette et al. 2007). Coating nisin in alginate film successfully reduced the levels of *S. aureus*. This elucidated the need for encapsulating nisin before it is used in a meat substance.

6.2 Nisin Activity

Nisin was measured throughout the 365 day study and was active during the entire 365-day period in all three sandwiches and under all three storage conditions (Tables 2-7) as indicated by the continued reduction or inhibition of *Salmonella*, *Listeria* and *Staphylococcus* strains. The three types of bacteria (*L. brevis*, *M. luteus*, and *S. aureus)* used to measure the zone of clearance had clearance present in each replicate throughout the experiment. The amount of clearance per bacterium differed in part to specificity of the study and laboratory contamination. Although the exact amount of nisin cannot be determined, the continual presence of nisin over time can be.

A linear regression model was used to determine the amount of nisin present in the sandwiches. The amount of bacterial inhibition was measured for each standard concentration, and a simple linear regression was developed to determine the relationship between the amount of nisin present and the average zone of inhibition. This model predicted the amount of nisin present in the samples by utilizing the relationship between the amount of bacterial inhibition observed in the known standard concentrations (Figure 10) and the amount of bacterial inhibition observed in the sandwiches (Figure 11). This relationship was use to estimate the concentration of nisin in the unknown samples (Figures 2-7).

The BBQ sandwich had lower nisin activity than the two formulations of Italian sandwiches and could be due to the type of meat used in the sandwiches. This may be supported in a study conducted by Cutter and Siragusa (1998), nisin was not effective when directly added to beef. Additionally, in a study conducted by Rose et al. (1999), it was

determined that nisin was successful not at inhibiting pathogens in fresh eye of round beef and juices. The meat was soaked for one hour at 4°C and the purge was combined to the same amount of nisin and held overnight at 4°C. Instead, the addition of nisin in a vessel is needed to ensure nisin's effectiveness. For example, the results revealed by Cutter and Siragusa (1998) whereby nisin was encapsulated in a meat binding called Fibramix to inhibit spoilage microorganisms. The results in the current study also correspond to those discovered by Scott and Taylor (1981), which determined that nisin is more effective at a pH of 6 or lower. The sandwiches used in our study, all containing a pH of 6.0 or lower, reduced the levels of the pathogens of concern.

6.3 Limitations and Complications

Many complications occurred throughout the bacterial assay and nisin assay. *Staphylococcus aureus* was present in the negative control samples causing baseline data to not be as reliable as desired. Positive control samples probably occurred during the hand-packing of the sandwiches. Approximately 30-50% of humans are carriers of *S. aureus*, carrying the pathogen on their skin, hair or in nasal passages (Forsythe, 2011). Although it was problematic having contaminated control samples, the prevalence of *S. aureus* declined overtime and growth was not observed, as in the inoculated samples obtained in the study.

Another complication to the bacterial assay was that there was background contamination on many plates. This was due to mold contamination caused by improper sealing of the MRE bags or the sachets failing to be effective. Initial *Listeria monocytogenes* plates had high levels of growth which could be attributed to mold as well as laboratory

contamination (Figure 10). The use of the fungicide pentachloronitrobenzene (PCNB)(Sigma Aldrich, category number P2205, United States) was used to inhibit growth of organisms other than *L. monocytogenes* later in the study on the MOX media. After using the fungicide, *L. monocytogenes* was easier to detect.

In the nisin assay, a variety of complications occurred. First, certain plates would fracture due to the well making process. This led to leakage of standards and samples throughout the plate, making the zone of inhibition measurement difficult. Second, the use of calipers proved to be an imprecise tool of measurement for bacterial inhibition due to operator variation. A standardized method for using the caliper was used and the same individual took the inhibition zone reading to ensure uniformity. Third, the standardized protocol for nisin quantification followed for this study was performed with liquid samples. This study used solid sandwiches and the sandwiches were diluted to achieve a liquid form so the material could be added to the wells. A dilution factor was not accounted for, as the dilution factor could not be added after nisin counts were achieved. This suggests that the amount of nisin present in the sandwiches was greater than found in the study, enhancing the amount of activity of nisin greater throughout the 365 day study.

6.4 Conclusion

In summary, the use of nisin in addition with multiple hurdles including pH, and reduced water activity effectively inhibited *S. aureus*, *S. enterica* and *L. monocytogenes* growth and caused pathogen reduction at 26.7, 37.7 and 48.8°C over 365 days. Although some pathogens were inhibited at slower rates than others, the pathogens were reduced

several log, and growth over time was not observed. Additionally, nisin activity was observed throughout the 365 days in each sandwich.

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TABLES AND FIGURES

Tables

Table 1. Water activity and pH of three types of MRE sandwiches

Sandwich Type	**a_W**	**pH**
BBQ	0.95	6
Italian $a_W = 0.92$	0.92	5.25
Italian $a_W = 0.90$	0.9	5

Table 2. Nisin concentrations (IU/ml) observed in MRE sandwiches at day 0 of storage predicted by comparing the zone of inhibition of three different organisms at standard concentrations to the zone of inhibition observed for each sandwich type

	Predicted Nisin Level, Day 0		
Type	***Staphylococcus aureus***	***Micrococcus luteus***	***Lactobacillus brevis***
BBQ	5 to 10 IU/ml	100 to 500 IU/ml	75 to 100 IU/ml
Italian $a_W = 0.90$	5 to 10 IU/ml	200 IU/ml	75 to 200 IU/ml
Italian $a_W = 0.92$	5 to 10 IU/ml	200 IU/ml	100 IU/ml

Table 3. Nisin concentrations (IU/ml) observed in MRE sandwiches after 28 days of storage predicted by comparing the zone of inhibition of three different organisms at standard concentrations to the zone of inhibition observed for each sandwich type

		Predicted Nisin Level, Day 28		
Type	**Temperature °C**	***Staphylococcus aureus***	***Micrococcus luteus***	***Lactobacillus brevis***
BBQ	26.7	0 IU/ml	Between 100 and 200 IU/ml	50 to 100 IU/ml
Italian a_W =0.90	26.7	0 to 25 IU/ml	100 IU/ml	25 to 75 IU/ml
	37.7	0 to 10 IU/ml	200 IU/ml	50 to 300 IU/ml
	48.8	0 IU/ml	Between 100 and 200 IU/ml	50 to 200 IU/ml
Italian 0.92	26.7	0 IU/ml	100 to 500 IU/ml	50 to 200 IU/ml
	37.7	0 to 10 IU/ml	Between 100 and 200 IU/ml	50 to 200 IU/ml
	48.8	0 IU/ml	200 IU/ml	25 to 100 IU/ml

Table 4. Nisin concentrations (IU/ml) observed in MRE sandwiches after 56 days of storage predicted by comparing the zone of inhibition of three different organisms at standard concentrations to the zone of inhibition observed for each sandwich type

		Predicted Nisin Level, Day 56		
Type	**Temperature °C**	***Staphylococcus aureus***	***Micrococcus luteus***	***Lactobacillus brevis***
BBQ	26.7	5 to 10 IU/ml	5 to 10 IU/ml	75 to 100 IU/ml
Italian $a_W = 0.90$	26.7	5 to 10 IU/ml	5 to 10 IU/ml	200 to 400 IU/ml
	37.7	5 to 10 IU/ml	5 to 10 IU/ml	25 to 100 IU/ml
	48.8	5 to 10 IU/ml	5 to 10 IU/ml	200 to 400 IU/ml
Italian $a_W = 0.92$	26.7	5 to 10 IU/ml	5 to 10 IU/ml	200 to 500 IU/ml
	37.7	5 to 10 IU/ml	5 to 10 IU/ml	200 IU/ml
	48.8	5 to 10 IU/ml	5 to 10 IU/ml	200 IU/ml

Table 5. Nisin concentrations (IU/ml) observed in MRE Sandwiches after 84 days of storage predicted by comparing the zone of inhibition of three different organisms at standard concentrations to the zone of clearing observed for each sandwich type

		Predicted Nisin Level, Day 84		
Sandwich Type	**Temperature °C**	***Staphylococcus aureus***	***Micrococcus luteus***	***Lactobacillus brevis***
BBQ	26.7	5 IU/ml	200 IU/ml	75 to 100 IU/ml
Italian $a_W = 0.90$	26.7	5 IU/ml	200 IU/ml	75 to 300 IU/ml
	37.7	5 IU/ml	200 IU/ml	75 to 400 IU/ml
	48.8	5 IU/ml	100 to 200 IU/ml	75 to 200 IU/ml
Italian $a_W = 0.92$	26.7	5 IU/ml	100 to 200 IU/ml	75 to 200 IU/ml
	37.7	*	100 IU/ml	50 to 200 IU/ml
	48.8	5 IU/ml	75 to 100 IU/ml	50 to 100 IU/ml

* Samples were contaminated and amount of nisin could not be determined.

Table 6. Nisin concentrations (IU/ml) observed in MRE sandwiches at day 156 of storage predicted by comparing the zone of inhibition of three different organisms at standard concentrations to the zone of inhibition observed for each sandwich type

		Predicted Nisin Level, Day 156		
Type	**Temperature °C**	***Staphylococcus aureus***	***Micrococcus luteus***	***Lactobacillus brevis***
BBQ	26.7	10**	200 to 300 IU/ml	25 to 500 IU/ml
Italian $a_W = 0.90$	26.7	10**	200 to 300 IU/ml	100 to 200 IU/ml
	37.7	10**	50 to 300 IU/ml	25 to 75 IU/ml
	48.8	10**	75 to 100 IU/ml	10 to 500 IU/ml
Italian $a_W = 0.92$	26.7	10**	200 IU/ml	50 to 100 IU/ml
	37.7	10**	200 to 300 IU/ml	50 to 75 IU/ml
	48.8	10**	100 to 200 IU/ml	25 to 100 IU/ml

**All predicted nisin levels were at 10 IU/ml.

Table 7. Nisin concentrations (IU/ml) observed in MRE sandwiches at day 365 of storage predicted by comparing the zone of inhibition of three different organisms at standard concentrations to the zone of inhibition observed for each sandwich type

		Predicted Nisin Level, Day 365		
Sandwich Type	**Temperature °C**	***Staphylococcus aureus***	***Micrococcus luteus***	***Lactobacillus brevis***
BBQ	26.7	5 to 50 IU/ml	200 to 400 IU/ml	75 to 100 IU/ml
Italian $a_W = 0.90$	26.7	5 to 50 IU/ml	200 to 500 IU/ml	200 to 300 IU/ml
	37.7	5 to 25 IU/ml	50 to 200 IU/ml	25 to 100 IU/ml
	48.8	5 to 25 IU/ml	50 to 100 IU/ml	50 to 100 IU/ml
Italian $a_W = 0.92$	26.7	5 to 25 IU/ml	300 to 500 IU/ml	100 to 200 IU/ml
	37.7	5 to 10 IU/ml	50 IU/ml	25 to 50 IU/ml
	48.8	5 to 25 IU/ml	50 to 100 IU/ml	25 to 75 IU/ml

Table 8. Representation of the Cronbach alpha for each variable in the theory of planned behavior survey to illustrate instrument reliability

	Cronbach's Alpha
Behavioral Control	0.788
Subjective Norms	0.809
Perceived Behavioral Controls	0.788
Intent	0.943

Table 9. Results of the paired T-Test comparing pre-test and post-test variables of the theory of planned behavior

Question	Type	Pre-Test Mean	Post-test Mean	Standard Deviation Pre-Test	Standard Deviation Post-Test	P-value (2 tail)
Behavioral Beliefs						
Food safety is important	Attitude Question 1	6.88	6.88	0.332	0.332	1
Foodborne training is important to limit foodborne illness	Attitude Question 2	6.76	6.94	0.437	0.243	0.083
Prerequiste programs are important to the HACCP plan	Attitude Question 5	5.06	6.53	1.088	1.007	0.001
Prerequiste programs are important to limit foodborne illness	Attitude Question 6	5.76	6.71	1.2	0.588	0.005
Food safety is not important	Attitude Question 7	1.06	1.06	0.243	0.243	0
Personnel training is not important for limiting foodborne illness	Attitude Question 8	1.18	1.06	0.393	0.243	0.163
Prereq programs are not important to the HACCP plan	Attitude Question 10	2.47	1.29	1.375	0.47	0.002
Prereq programs are not important in reducing foodborne illness	Attitude Question 11	2.18	1.24	1.237	0.437	0.004
Perceived Control Beliefs						
Risk factors for food posioning can be controlled	Control Question 1	5.59	5.82	1.66	1.19	0.332
My actions as work can prevent customers from contracting food posioning	Control Question 2	5.94	6.18	1.3	1.02	0.216
I am able to diligently conduct safety tasks in my workplace	Control Question 3	6	6.29	0.94	0.686	0.096
Costumers could contract food poisioning from my work regardless of how diligent I am about food safety	Control Question 4	4.35	4.29	1.58	2.09	0.921
I have control over whether someone contracts food poisioning from a place you currently work or will work in the future	Control Question 5	4.94	4.24	1.2	1.2	0.176
Carrying out food safety behaviors at every occurance would be	Control Question 6	5.41	5.71	1.17	1.11	0.311
If I wanted to carry out food safety behaviors at every occurance I would be able to	Control Question 7	5.41	5.65	1.18	0.99	0.387
Normative Beliefs						
My friends	Normative Question 1	5.46	5.76	1.52	1.15	1
My family	Normative Question 2	6	6	1.27	1.06	1
My boss	Normative Question 3	6	6.24	1.12	0.664	0.216
My coworkers	Normative Question 4	5.82	6.18	1.33	0.728	0.138
My teacher	Normative Question 5	5.94	6.59	1.3	0.618	0.06
My fellow students	Normative Question 6	5.82	5.82	1.33	1.13	1
Intent Questions						
Implement prerequisite programs in your future facility	Intent Question 1	5.82	6.24	1.01	1.03	0.13
Keep up to date with requirements of prerequiste programs	Intent Question 2	5.94	6.29	0.9	0.77	0.21
Evaluate prerequiste programs of the food service or food processing facilities you enter	Intent Question 3	5.65	6.24	0.1	0.75	0.04
Practice food safety behaviors in my current or future food service or processing facility	Intent Question 4	6	4.24	1.3	1.9	0.001

Table 10. Average mean of standard nisin concentrations developed for standard curve and the confidence limits on day 365

	Staphylococcus aureus			***Micrococcus luteus***			***Lactobacillus brevis***		
Standard Nisin Concentration	**Mean**	**Confidence Limit for Mean**		**Mean**	**Confidence Limit for Mean**		**Mean**	**Confidence Limit for Mean**	
500	1.05	1.04	1.07	1.26	1.23	1.28	1.48	1.44	1.51
400	1.05	1.03	1.06	1.24	1.22	1.26	1.45	1.42	1.49
300	1.04	1.02	1.05	1.22	1.2	1.24	1.42	1.39	1.45
200	1.03	1.01	1.04	1.19	1.17	1.21	1.38	1.35	1.41
100	1.01	0.99	1.02	1.14	1.12	1.15	1.3	1.28	1.33
75	0.99	0.98	1.01	1.11	1.1	1.13	1.27	1.25	1.29
50	0.98	0.97	0.99	1.08	1.07	1.1	1.23	1.2	1.25
25	0.96	0.95	0.97	1.03	1.02	1.05	1.15	1.13	1.18
10	0.94	0.92	0.95	0.96	0.94	0.98	1.02	1.02	1.08
5	0.91	0.90	0.93	0.91	0.89	0.93	0.98	0.94	1.02
0	0.86	0.84	0.89	0.79	0.76	0.83	0.8	0.75	0.86

Table 11. Average mean nisin concentration and confidence limits for BBQ sandwich and Italian sandwiches containing different water activities and pH concentrations on Day 365

		Staphylococcus aureus			***Micrococcus luteus***			***Lactobacillus brevis***		
Sandwich Type	**Temp**	**Mean**	**Confidence Limit**		**Mean**	**Confidence Limit**		**Mean**	**Confidence Limit**	
BBQ	80	0.94	0.91	0.97	1.21	1.18	1.23	1.3	1.26	1.33
Italian a_W=0.90 pH=5.0	80	0.94	0.91	0.98	1.23	1.19	1.26	1.37	1.33	1.41
	100	0.93	0.91	0.96	1.14	1.09	1.18	1.23	1.15	1.31
	-	-	-	-	-	-	-	-	-	-
	120	0.93	0.901	0.96	1.12	1.09	1.16	1.24	1.18	1.29
Italian a_W=0.92 pH=5.25	80	0.94	0.91	0.97	1.23	1.21	1.24	1.34	1.32	1.36
	100	0.93	0.91	0.94	1.08	1.06	1.11	1.18	1.14	1.22
	120	0.94	0.91	0.9	1.11	1.07	1.15	1.23	1.18	1.27

Figures

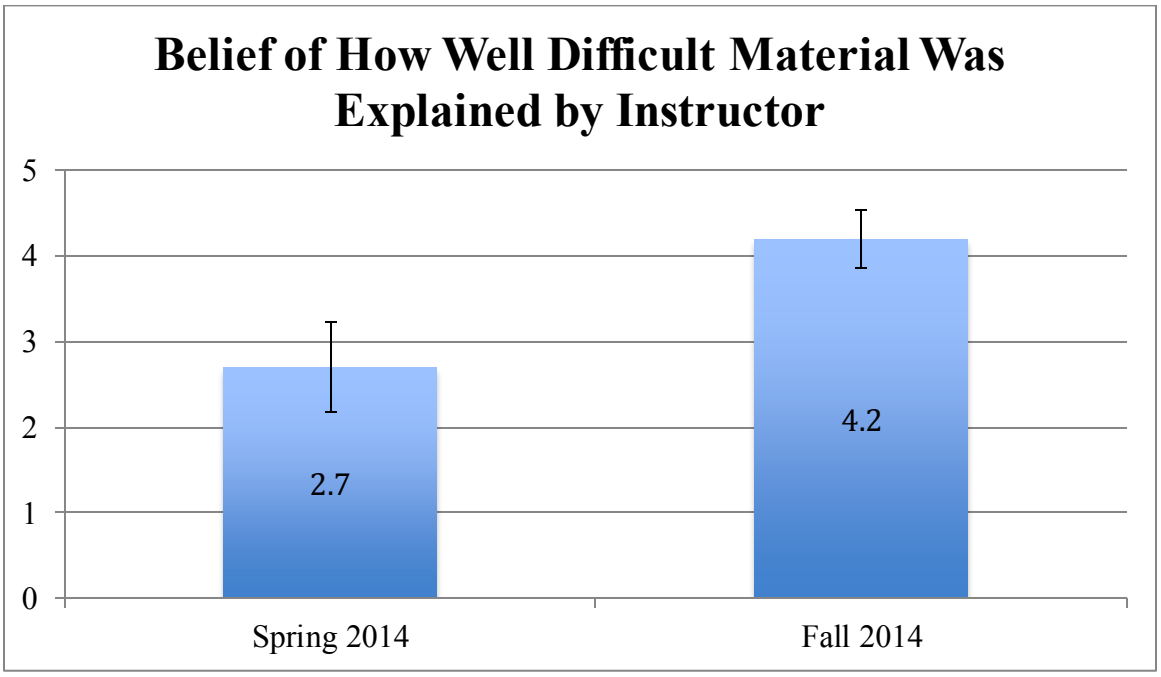

Figure 1. Average rating of Spring 2014 and Fall 2015 Introductory to HACCP course participants about their belief on how well the information taught was explained (5-very well; 0-not well)

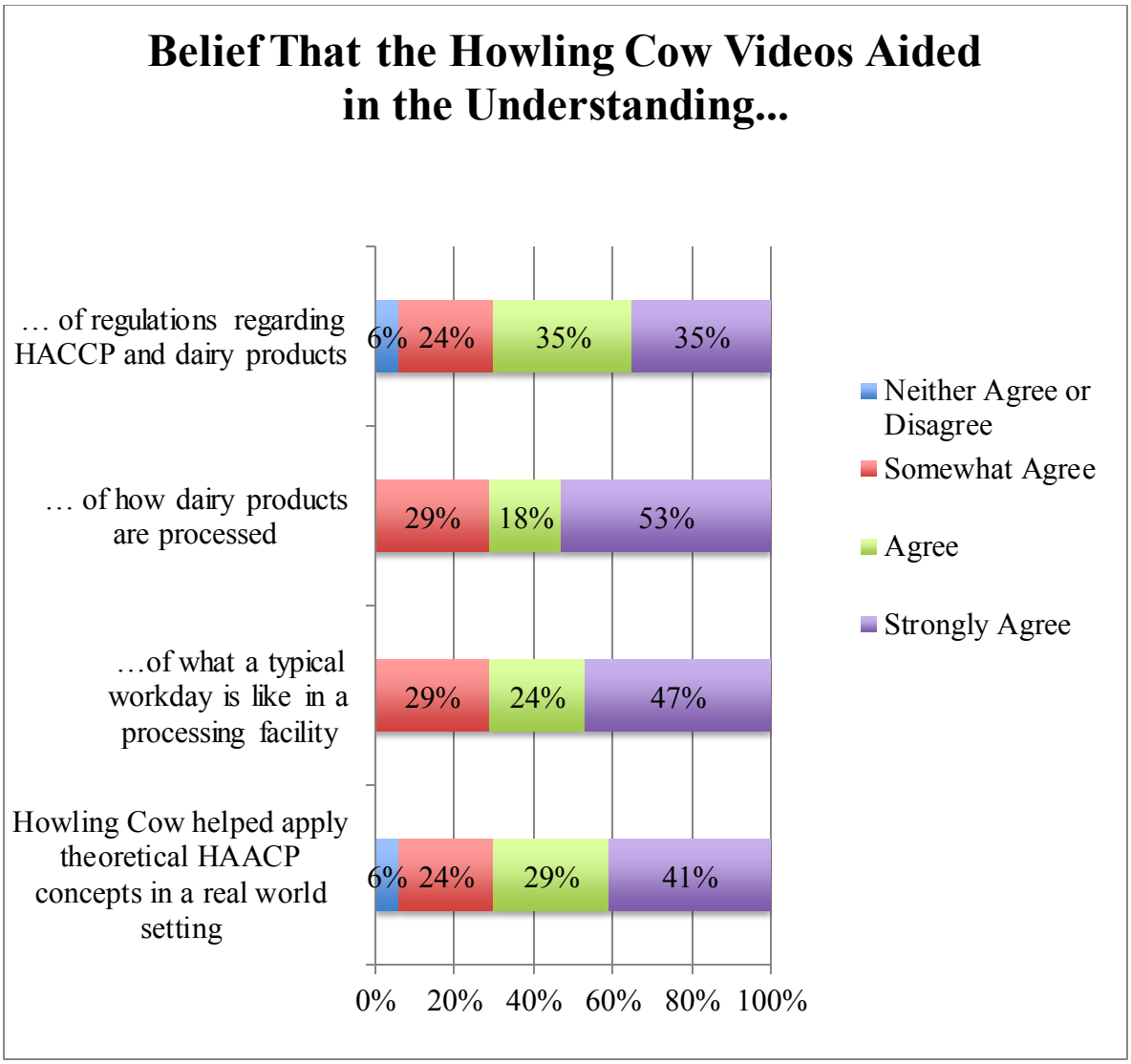

Figure 2. Students determination of how much the Howling Cow videos aided in their understanding of certain learning concepts

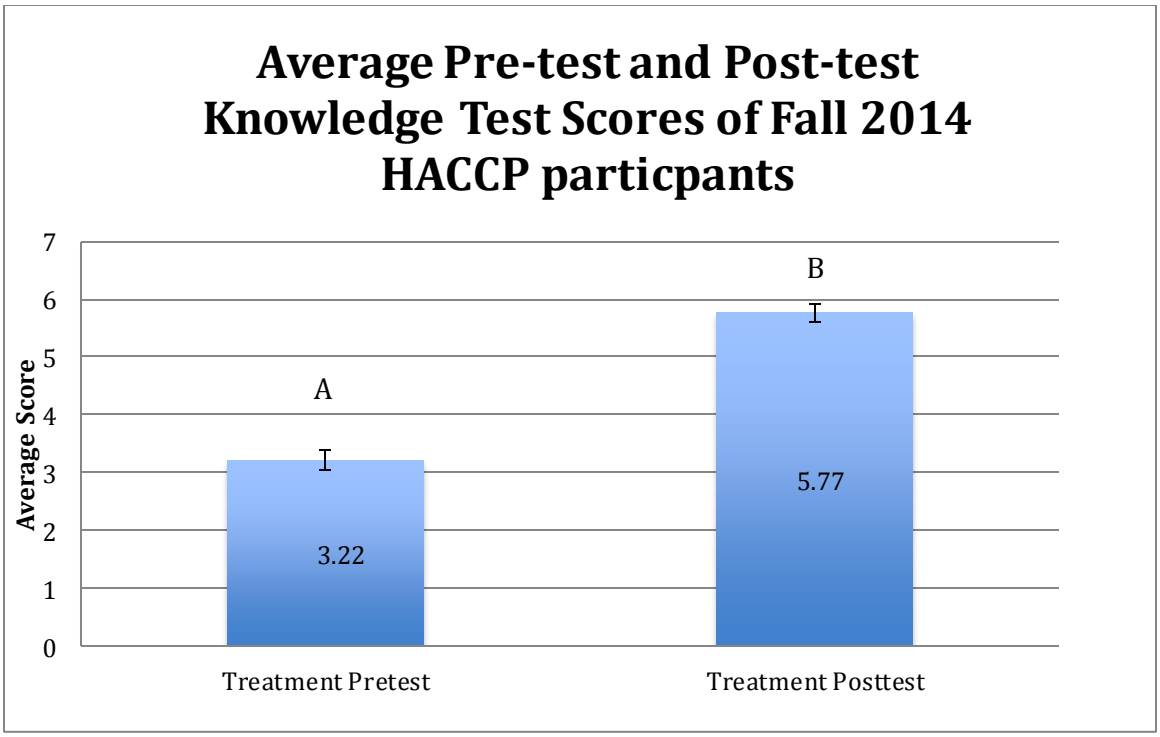

Figure 3. Knowledge score of pre-test and post-test of participants (significant difference denoted in letter change)

Best possible score was 10

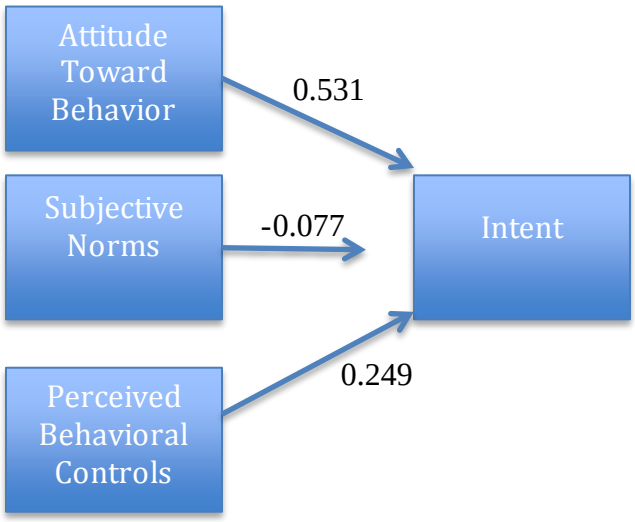

Figure 4. Pathways analysis using the theory of planned behavior to predict the how these variables affect behavioral intention

Pathways coefficient are represented in numbers present

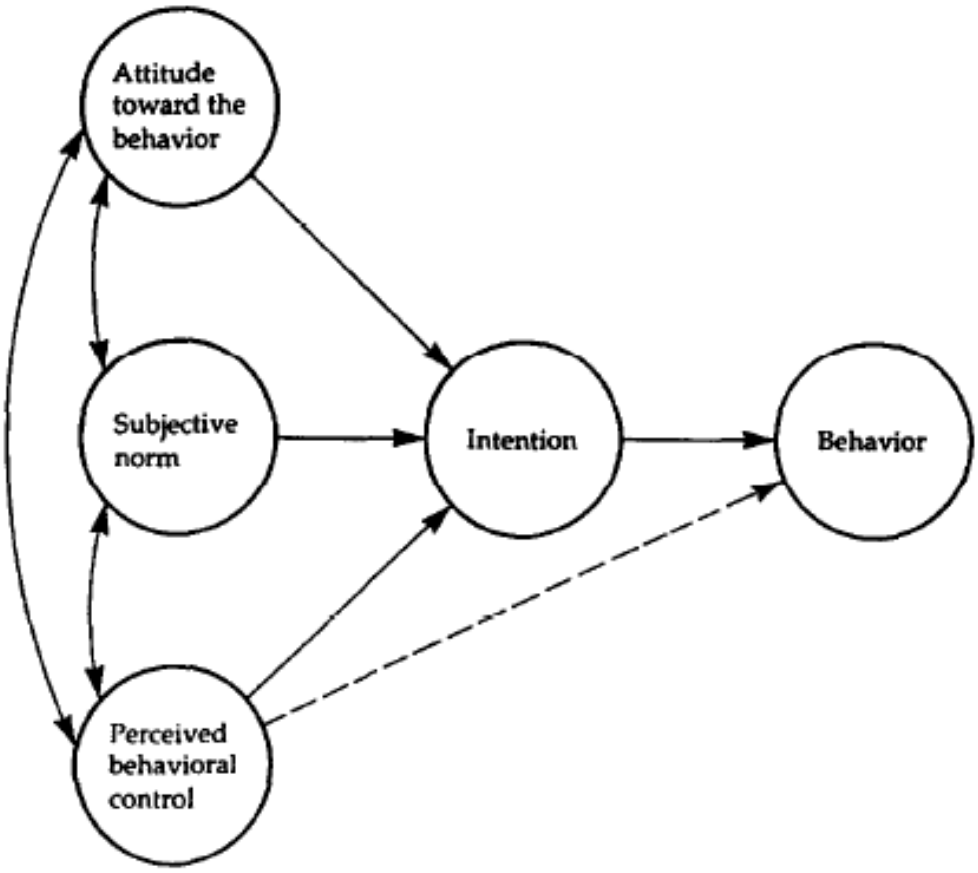

FIG. 1. Theory of planned behavior.

Figure 5. Schematic illustration of the theory of planned behavior model.

Adapted from Ajzen, 1991

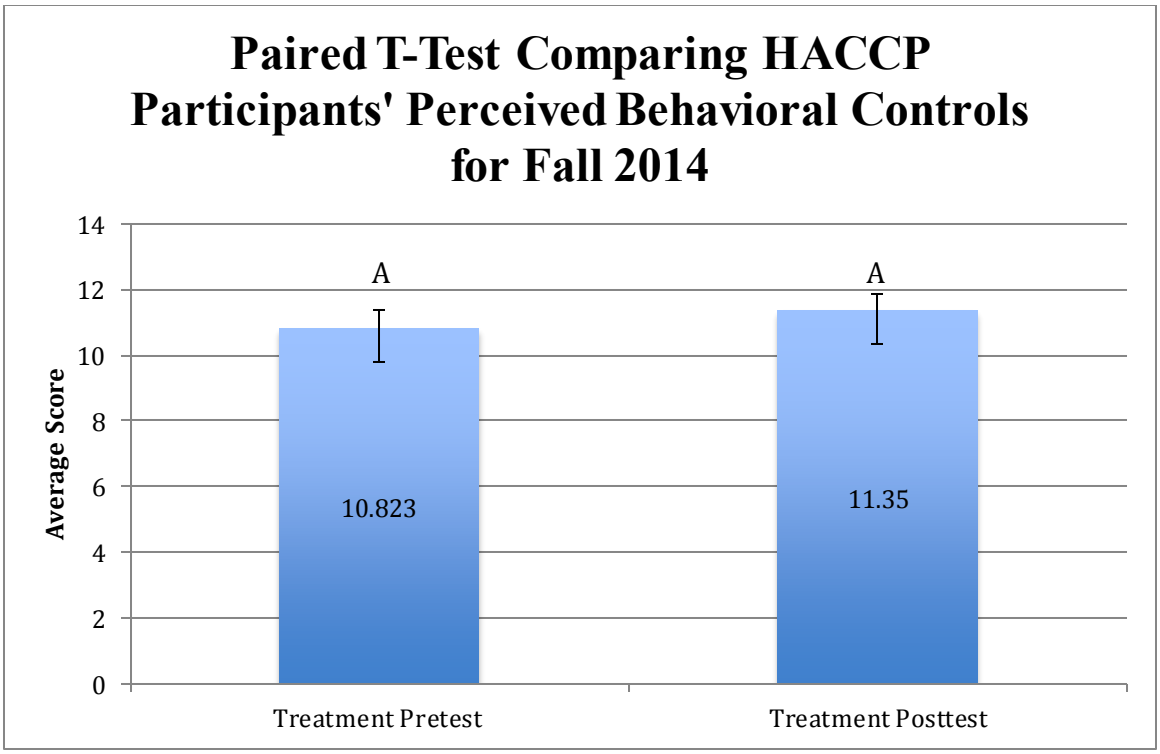

Figure 6. Average pre-test and post-test score of participants' perceived behavioral controls (no significant difference observed)

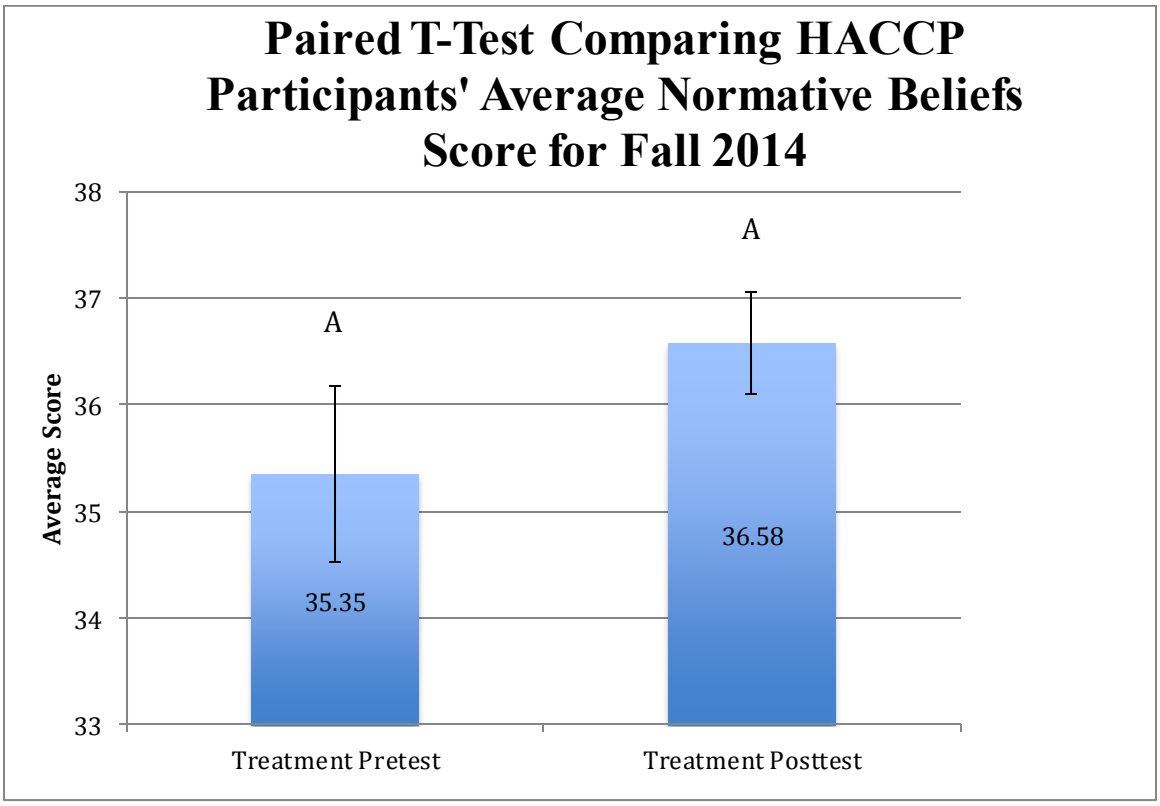

Figure 7. Average pre-test and post-test score of participants' normative beliefs (no significant difference observed)

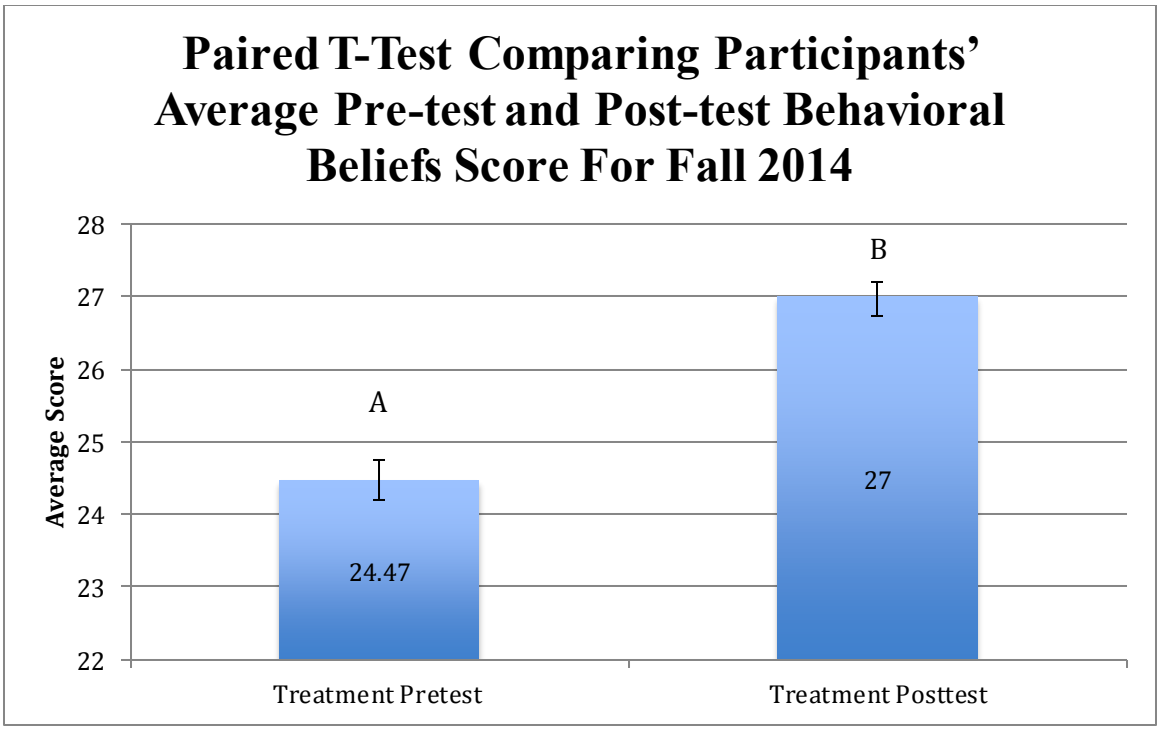

Figure 8. Average pre-test and post-test score of participants' behavioral beliefs (significant difference denoted by letter change)

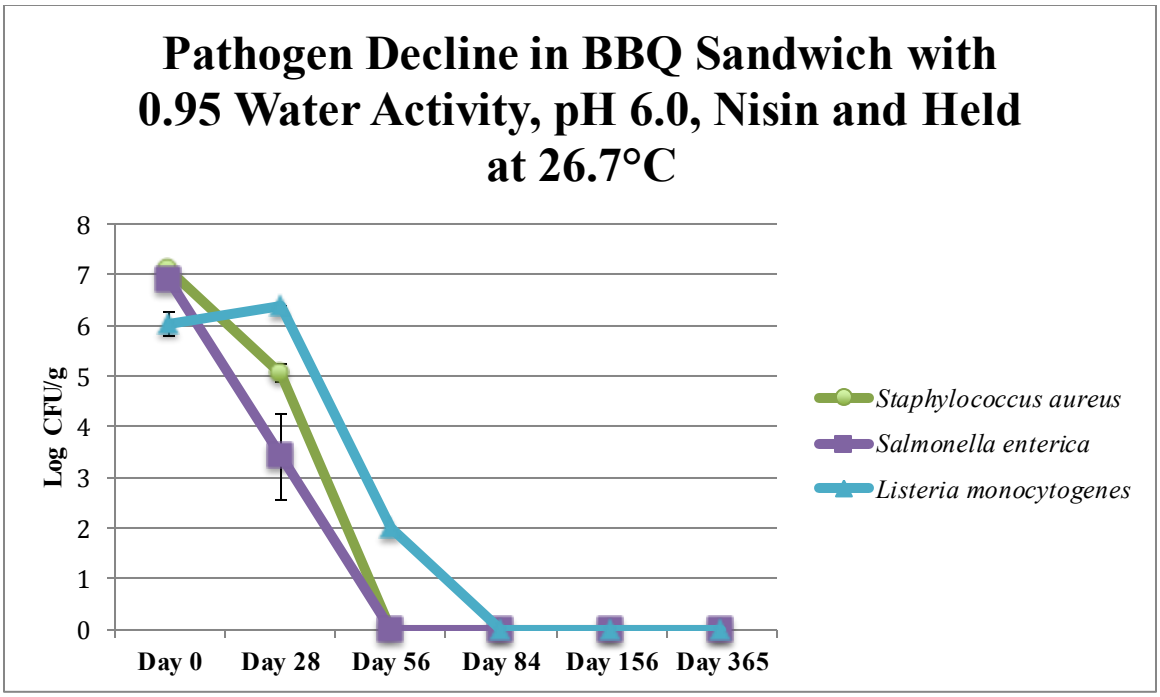

Figure 9. Pathogen decline over course of 365 days in BBQ sandwich held at 26.7°C

Staphylococcus aureus control (not pictured) had one replicate on day 28 at log 6.17 CFU/g

Listeria monocytogenes control (not pictured) had one replicate on day 28 at log 2.18 CFU/g

Staphylococcus aureus had one replicate at log 5.15 CFU/g on day 156

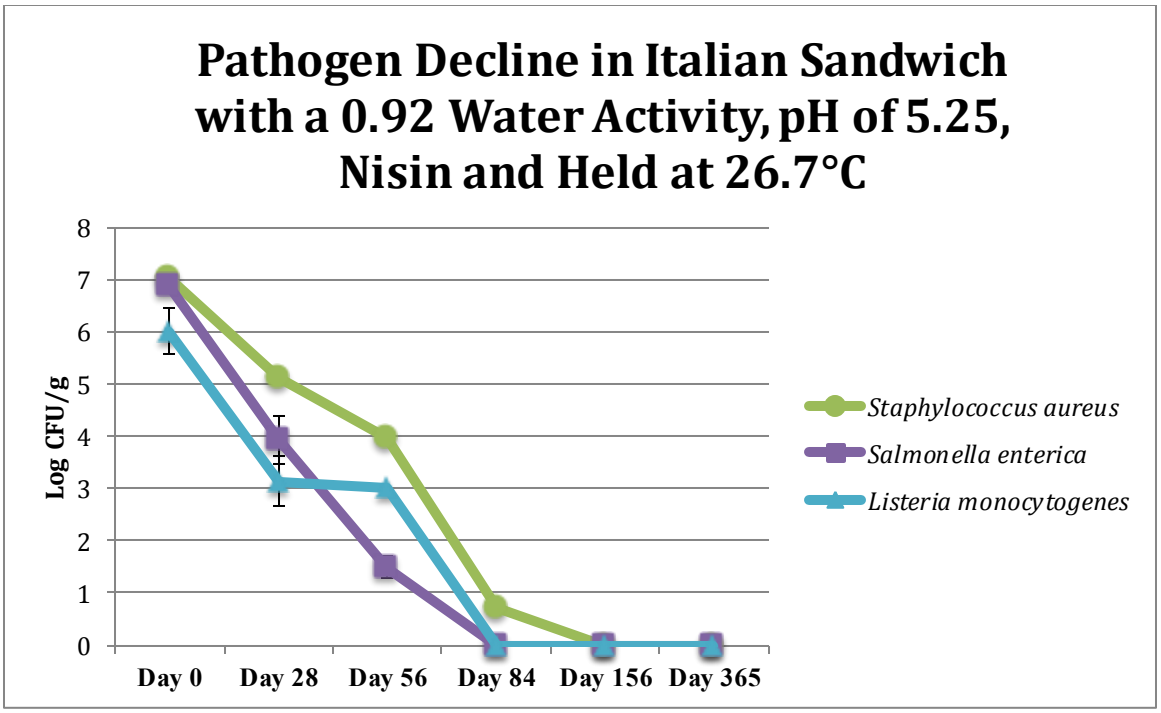

Figure 10. Pathogen decline over course of 365 days in Italian sandwich with a water activity of 0.92 and held at 26.7°C

Staphylococcus aureus control (not pictured) had positive results on day 28 at log 5.06 CFU/g and again on day 56 at log 5.23 CFU/g

Staphylococcus aureus had growth on day 156 at log 3.94 CFU/g, but one replicate was nondetectable. Day 365 had one replicate at log 2.58 CFU/g

Listeria monocytogenes had one replicate on day 156 at log 9.84 CFU/g due to laboratory contamination

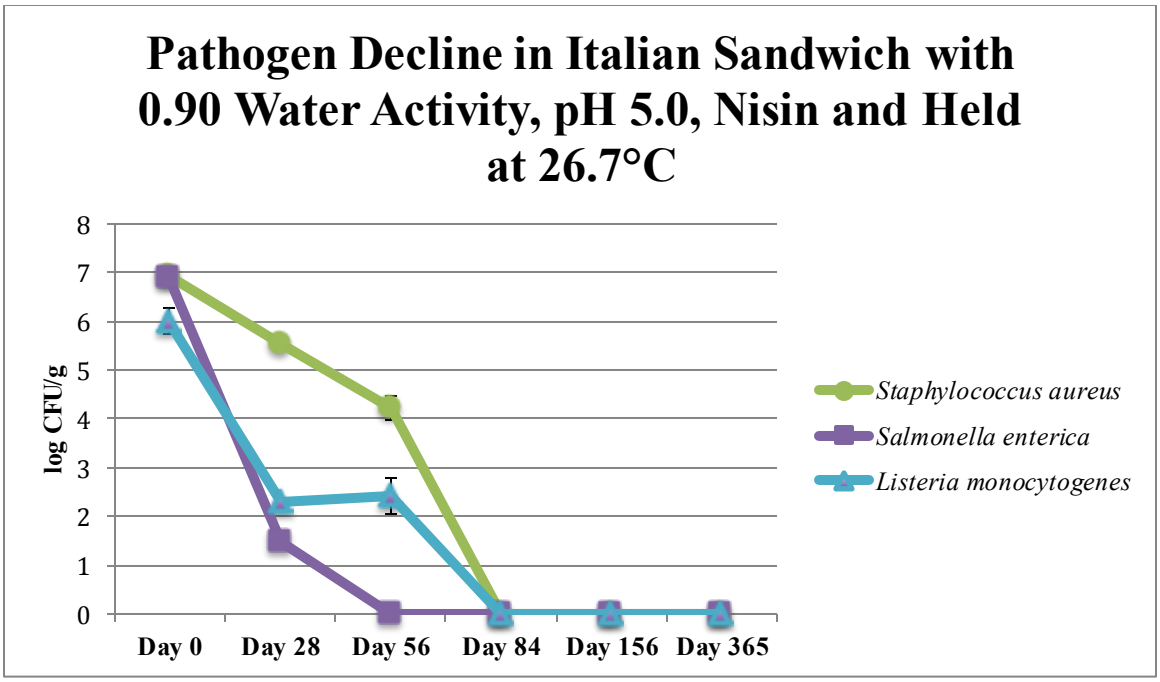

Figure 11. Pathogen decline over course of 365 days in Italian sandwich with a water activity of 0.90 and held at 26.7°C

Staphylococcus aureus control (not pictured) had positive results on day 28 at log 4.65 CFU/g

Staphylococcus aureus had one replicate on day 84 at log 2.54 CFU/g and day 365 had one replicate at log 3.26 CFU/g

Listeria monocytogenes had one replicate on day 84 at log 2.60 CFU/g

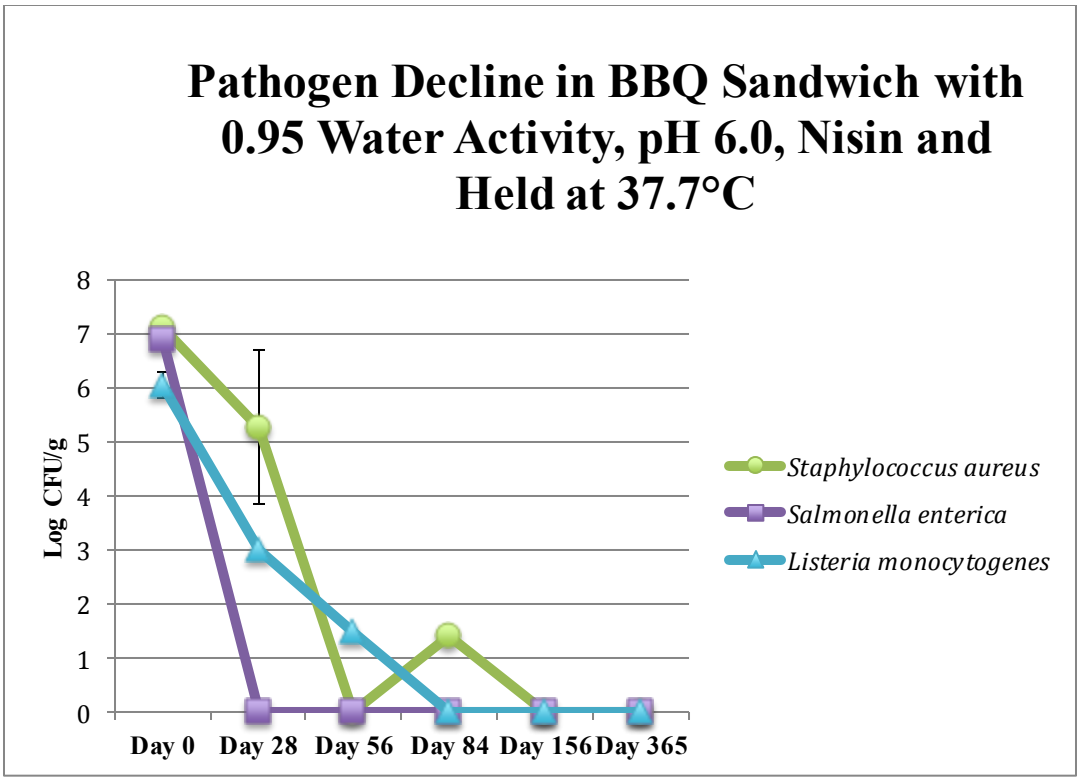

Figure 12. Pathogen decline over course of 365 days in BBQ sandwich and held at 37.7°C Controls (not pictured) were undetectable

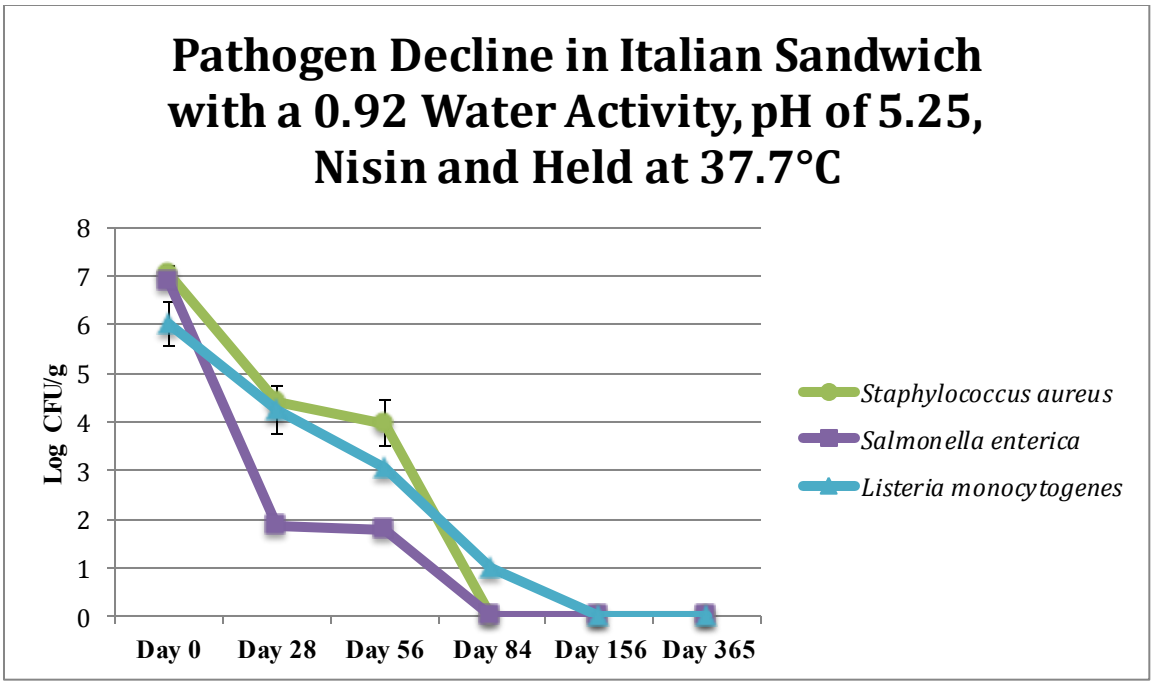

Figure 13. Pathogen decline over course of 365 days in Italian sandwich with a water activity of 0.92 and held at 37.7°C

Staphylococcus aureus control (not pictured) had one replicate peak on day 28 at log 6.66 CFU/g and another replicate peak on day 84 at log 3.03 CFU/g

Listeria monocytogenes control (not pictured) had one replicate on day 84 at log .84 CFU/g

Staphylococcus aureus had one detectable replicate on day 156 at log 4.30 CFU/g

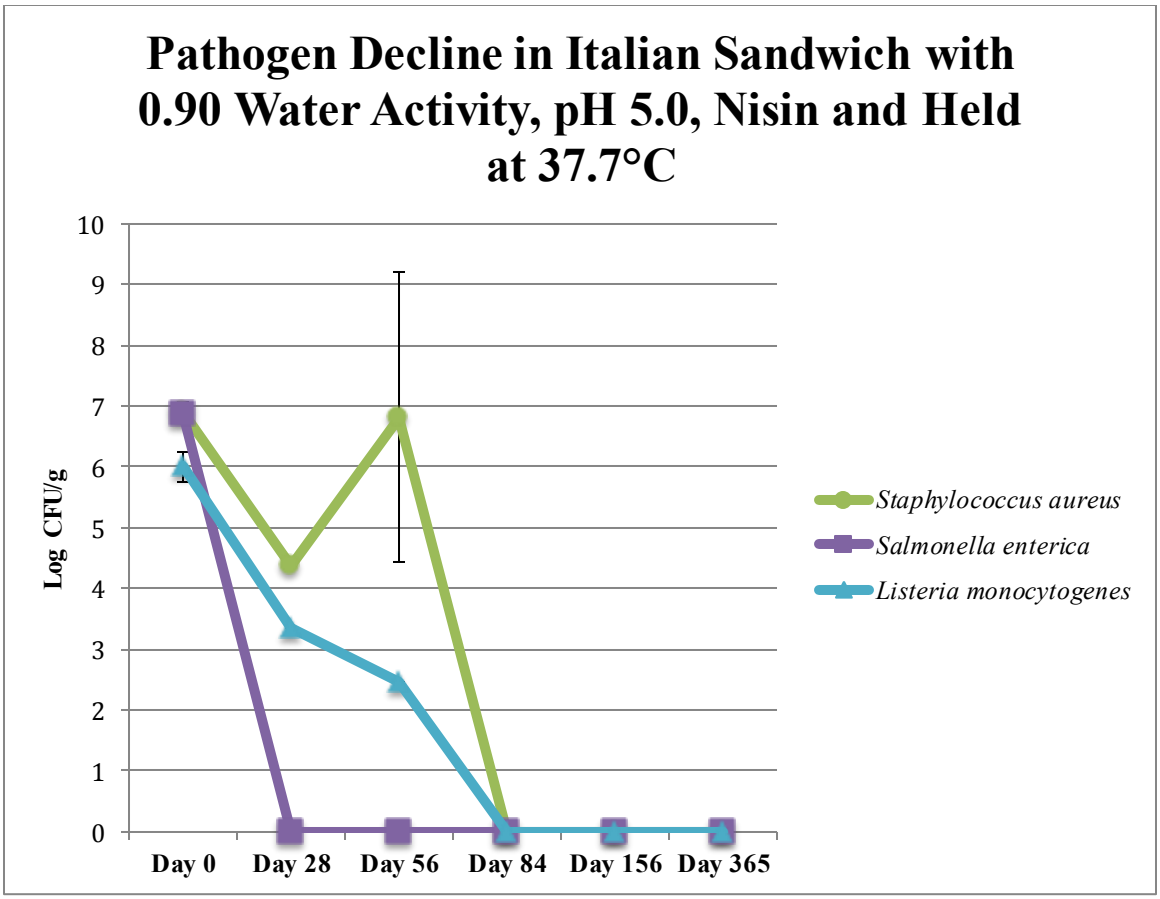

Figure 14. Pathogen decline over course of 365 days in Italian sandwich with a water activity of 0.90 and held at 37.7°C

Listeria monocytogenes control (not pictured) had one replicate on day 84 at log 3.73 CFU/g

Staphylococcus aureus had one positive replicate on day 156 at log 3.15 CFU/g

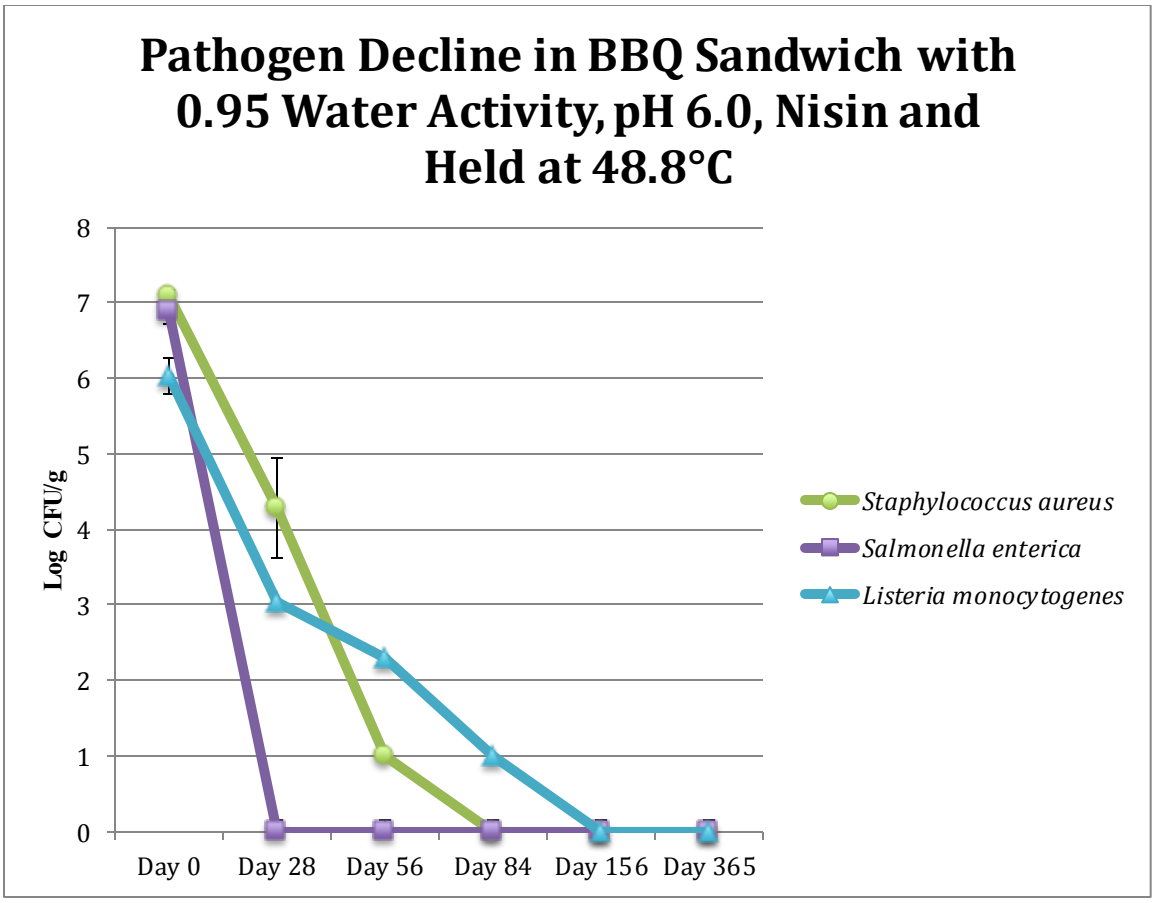

Figure 15. Pathogen decline over course of 365 days in BBQ sandwich held at 48.8°C *Staphylococcus aureus* control (not pictured) had one replicate peak on day 28 at log 6.66 CFU/g, another replicate peak on day 56 at 3.03 CFU/g and another replicate on day 84 at log 2.3 CFU/g

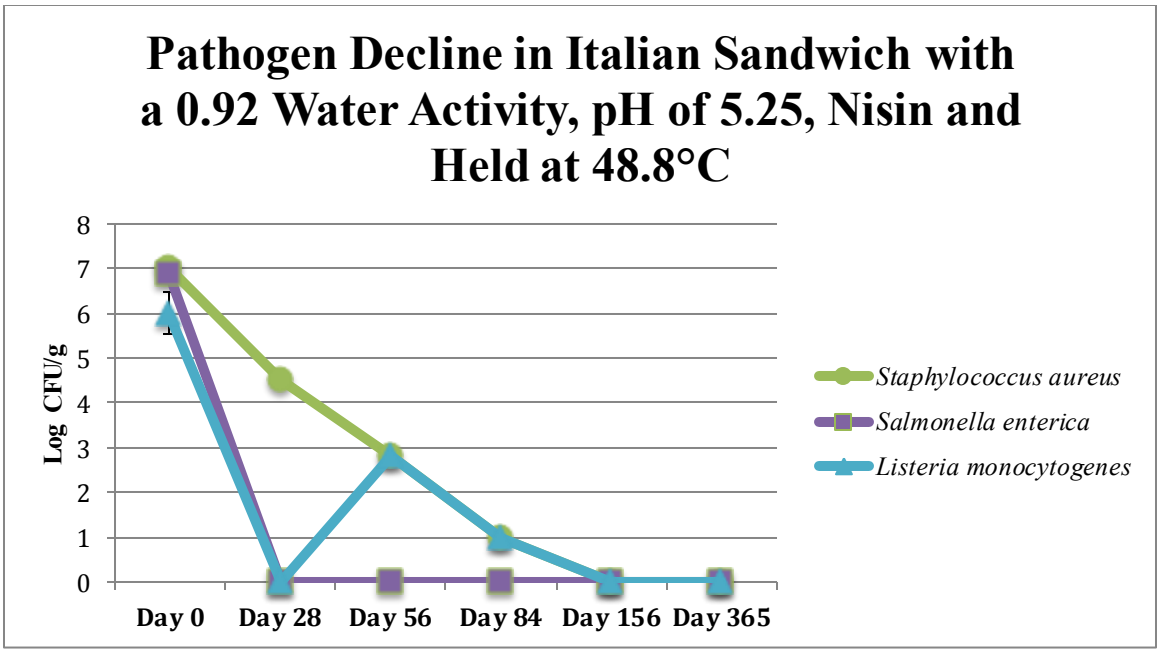

Figure 16. Pathogen decline over course of 365 days in Italian sandwich with a water activity of 0.92 and held at 48.8°C

Staphylococcus aureus control (not pictured) had one replicate peak on day 28 at log 6.73 CFU/g, and another replicate peak on day 56 at log .3 CFU/g

Staphylococcus aureus had one replicate on day 156 at log 3.81 CFU/g

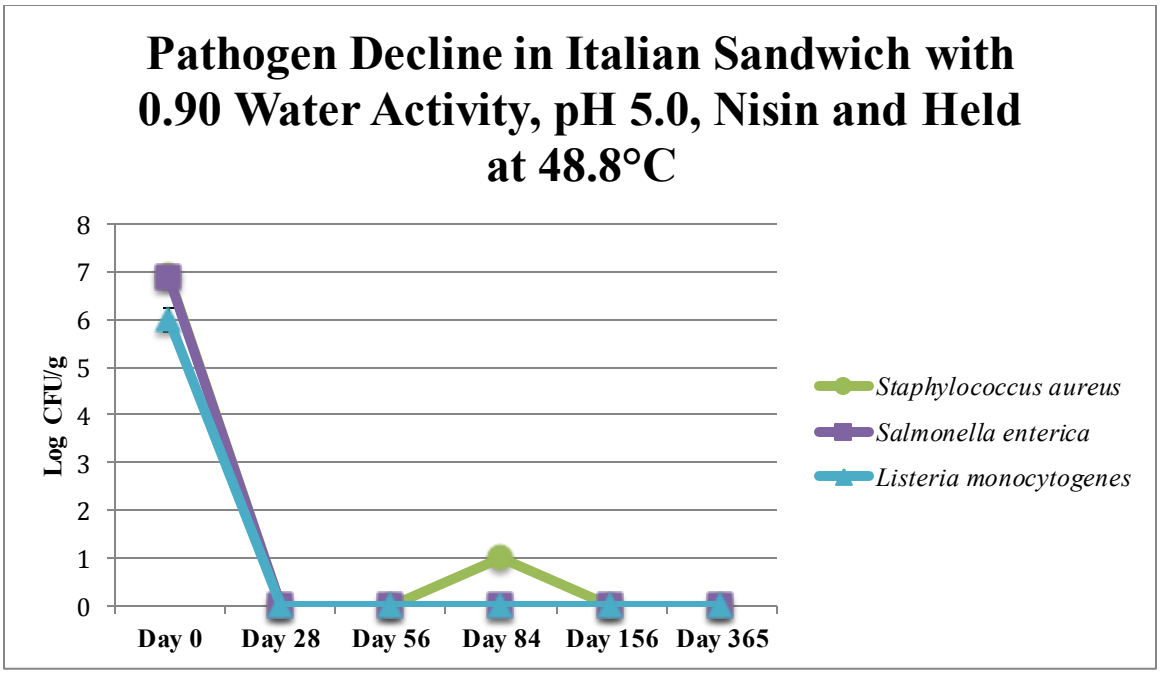

Figure 17. Pathogen decline over course of 365 days in Italian sandwich with a water activity of 0.90 and held at 48.8°C

Staphylococcus aureus control (not pictured) had one replicate peak on day 28 at log 2.77 CFU/g

Figure 18. Introduction to HACCP Post-Test Survey

Q1 Please indicate your level of agreement with the following statements:

	Strongly Disagree (1)	Disagree (2)	Somewhat Disagree (3)	Neither Agree nor Disagree (4)	Somewhat Agree (5)	Agree (6)	Strongly Agree (7)
Food safety is important (1)	❍	❍	❍	❍	❍	❍	❍
Personnel training is important to limiting foodborne illness (2)	❍	❍	❍	❍	❍	❍	❍
GMP's are more important than HACCP (3)	❍	❍	❍	❍	❍	❍	❍
GMP's and SSOPs are equally important (4)	❍	❍	❍	❍	❍	❍	❍
Prerequisite programs are important to the HACCP plan (5)	❍	❍	❍	❍	❍	❍	❍
Prerequisite programs are necessary to reduce foodborne illness (6)	❍	❍	❍	❍	❍	❍	❍

Figure 18. (Continued)

Food safety is not important (7)	❍	❍	❍	❍	❍	❍	❍
Personnel training is not important to limiting food borne illness (8)	❍	❍	❍	❍	❍	❍	❍
HACCP is more important than GMP's (9)	❍	❍	❍	❍	❍	❍	❍
Prerequisite programs are not important to the HACCP plan (10)	❍	❍	❍	❍	❍	❍	❍
Prerequisite programs are not important in reducing food borne illness (11)	❍	❍	❍	❍	❍	❍	❍

Q34 What are the important things you can do, when preparing or handling food at work, in order to prevent food poisoning? (please list as many things as you can)

Q35 Please list the advantages to carrying out these behaviors at every appropriate work occasion?

Q36 Please list the disadvantages to carrying out these behaviors at every appropriate work occasion?

Figure 18. (Continued)

Q37 What, if anything, would encourage you or make it easier for you, to carry out these behaviors at every appropriate work occasion?

Q38 What, if anything, would discourage you or make it harder for you, to carry out these behaviors at every appropriate work occasion?

Q39 Please indicate how much risk is associated with the following questions. Please rank with 1 being absolutely no risk, 4 being neutral and 7 being very risky.

Figure 18. (Continued)

	1 (1)	2 (2)	3 (3)	4 (4)	5 (5)	6 (6)	7 (7)
How much risk do you think is there of someone contracting food poisoning from food produced by the food industry? (1)	❍	❍	❍	❍	❍	❍	❍
How much risk do you think is there of someone contracting food poisoning from the place you work? (2)	❍	❍	❍	❍	❍	❍	❍
How much risk is there of someone contracting food poisoning from your home? (3)	❍	❍	❍	❍	❍	❍	❍

Figure 18. (Continued)

How much risk is there if food safety behaviors are preformed at a possible occasions? (4)	❍	❍	❍	❍	❍	❍	❍
How much risk is there if food safety behaviors are not preformed at all possible occasions? (5)	❍	❍	❍	❍	❍	❍	❍
How much risk is there if food safety behaviors are not preformed at all? (6)	❍	❍	❍	❍	❍	❍	❍

Figure 18. (Continued)

Q43 Please indicate how possible the following statements are.

	Strongly disagree (1)	Disagree (2)	Somewhat disagree (3)	Neither agree or disagree (4)	Somewhat agree (5)	Agree (6)	Strongly Agree (7)
Risk factors for food poisoning can be controlled (1)	❍	❍	❍	❍	❍	❍	❍
My actions at work can prevent customers from contracting food poisoning (2)	❍	❍	❍	❍	❍	❍	❍
I am able to diligently conduct food safety tasks in my workplace (3)	❍	❍	❍	❍	❍	❍	❍

Figure 18. (Continued)

Costumers could contract food poisoning from my work regardless of how diligent I am about food safety (4)	❍	❍	❍	❍	❍	❍	❍
I have control over whether someone contracts food poisoning from a place you currently work or will work in the future (5)	❍	❍	❍	❍	❍	❍	❍

Figure 18. (Continued)

Q41 Please indicate how likely it is that the following people think that it is important to implement food safety behaviors at ever possible occasion.

	Highly Unlikely (1)	Unlikely (2)	Somewhat Unlikely (3)	Neither Unlikely nor Likely (4)	Somewhat Likely (5)	Likely (6)	Very Likely (7)
My friends (1)	❍	❍	❍	❍	❍	❍	❍
My family (2)	❍	❍	❍	❍	❍	❍	❍
My boss (3)	❍	❍	❍	❍	❍	❍	❍
My coworkers (4)	❍	❍	❍	❍	❍	❍	❍
My teacher (5)	❍	❍	❍	❍	❍	❍	❍
My fellow students (6)	❍	❍	❍	❍	❍	❍	❍

Figure 18 (Continued)

Q42 Please indicate how possible the following statement would be.

	Not Possible At All (1)	Not Possible (2)	Somewhat Not Possible (3)	Neutral (4)	Somewhat Possible (5)	Possible (6)	Very possible (7)
Carrying out food safety behaviors at every occurrence would be (1)	❍	❍	❍	❍	❍	❍	❍

Q44 Please indicate how possible the following statement would be.

	Not Possible At All (1)	Not Possible (2)	Somewhat Not Possible (3)	Neutral (4)	Somewhat Possible (5)	Possible (6)	Very Possible (7)
If I wanted to carry out food safety behaviors at every occurrence I would be able to (1)	❍	❍	❍	❍	❍	❍	❍

Q24 FDA seafood HACCP regulation applies to the following except:

- ❍ Imitation crab dip (1)
- ❍ Caesar salad dressing with anchovies (2)
- ❍ Frogs (3)
- ❍ Salmon (4)

Figure 18. (Continued)

Q25 What are critical control points?

- ❍ Essential steps for the production of a safe finished product (1)
- ❍ Steps to take at all points of production to ensure a safe food product (2)
- ❍ Control measures to ensure a safe food measure (3)
- ❍ Steps where food safety can be controlled (4)

Q26 A CCP is designated at CCP B1. What does the B1 stand for?

- ❍ A hazard potential at the baking step (1)
- ❍ Burn Hazard (2)
- ❍ "B" grade hazard, which means moderate risk (3)
- ❍ Biological hazard (4)

Q27 According to current regulations in the U.S., who has the responsibility for development and implementation of HACCP plans?

- ❍ Industry (1)
- ❍ Codex (2)
- ❍ FDA, FSIS and USDA (3)
- ❍ NACFCM (4)

Figure 18. (Continued)

Q28 The FDA and USDA/FSIS require that HACCP records for frozen and shelf stable be held for at least:

- ❍ 6 months (1)
- ❍ 1 year (2)
- ❍ 2 years (3)
- ❍ 5 years (4)

Q29 What is typically the cause of HACCP system failures?

- ❍ Too many products and processes requiring HACCP plan development (1)
- ❍ Inadequate documentation of the HACCP plan (2)
- ❍ Inadequate training for all employees (3)
- ❍ Employee competency (4)

Q30 Prerequisite programs have no effect on the HACCP hazard analysis

- ❍ True (1)
- ❍ False (2)

Q31 For HACCP purposes, which of the following is considered a problem because it is aesthetically displeasing, as compared to a food safety hazard?

- ❍ Glass particles (1)
- ❍ Metal fragments (2)
- ❍ Wood splinters (3)
- ❍ 1 inch long hair (4)

Q32 What is the primary role of the HACCP team members?

- ❍ Perform monitoring and corrective actions procedures (1)
- ❍ Provide specific expertise (2)
- ❍ Offer an opinion when asked (3)
- ❍ Follow the directions of the FDA/USDA inspector (4)

Q33 The first preliminary task in developing a HACCP team is to:

- ❍ Appoint a HACCP coordinator and form a HACCP team (1)
- ❍ Describe the food product and how it is made (2)
- ❍ Construct a process flow diagram (3)
- ❍ Identify and evaluate the food safety hazards (4)

Figure 18. (Continued)

Q3 Please indicate your level of agreement with the following statements. After completion of this program I plan to:

	Very Unlikely (1)	Unlikely (2)	Somewhat Unlikely (3)	Neutral (4)	Somewhat Likely (5)	Likely (6)	Very Likely (7)
Implement prerequisite programs in your future facility (1)	❍	❍	❍	❍	❍	❍	❍
Keep up to date with requirements of prerequisite programs (2)	❍	❍	❍	❍	❍	❍	❍
Evaluate prerequisite programs of the food service or food processing facilities you enter (3)	❍	❍	❍	❍	❍	❍	❍
Practice food safety behaviors in my current or future food service or food processing facility (4)	❍	❍	❍	❍	❍	❍	❍

Figure 18. (Continued)

Q19 Please Indicate your age
- ❍ 18 or below (1)
- ❍ 19-20 (2)
- ❍ 21-23 (3)
- ❍ 24-26 (4)
- ❍ 27-28 (5)
- ❍ 29-31 (6)
- ❍ 32-34 (7)
- ❍ 35-37 (8)
- ❍ 38-40 (9)
- ❍ 41-42 (10)
- ❍ 43-49 (12)
- ❍ 50-59 (13)
- ❍ 59 or above (14)

Q20 Please indicate highest level of schooling
- ❍ Did not complete high school (1)
- ❍ Completed high school (2)
- ❍ Completed some college (3)
- ❍ Earned an Associates Degree (4)
- ❍ Earned a Bachelors Degree (5)
- ❍ Earned a Masters Degree (6)
- ❍ Earned a Ph.D. (7)
- ❍ Other (8) _____

Q21 Indicate employment status of best fit
- ❍ Currently unemployed (1)
- ❍ Student (2)
- ❍ Working professional (3)

If Working professional Is Selected, Then Skip To Click to write the question text

Q22 Please indicate which title best fits your current job title.
- ❍ Line worker (1)
- ❍ Shift manager or supervisor (2)
- ❍ QA manager or supervisor (3)
- ❍ QC manager or supervisor (4)
- ❍ Other (5) _____

Figure 18. (Continued)

Answer If Indicate employment status of best fit Working professional Is Not Selected

Q50 Are you interested in working in the food industry?

- ❍ Yes (1)
- ❍ No (2)
- ❍ Maybe (3)
- ❍ Other (4)

Answer If Indicate employment status of best fit Working professional Is Selected

Q23 How many years of experience do you have in the food industry?

- ❍ None (1)
- ❍ Less than 1 year (2)
- ❍ 1-2 years (3)
- ❍ 3-4 years (4)
- ❍ 5-6 years (5)
- ❍ 7-10 years (6)
- ❍ 11-15 years (7)
- ❍ 16-20 years (8)
- ❍ 21 years or more (9)

Figure 18. (Continued)

Q4 Please indicate your satisfaction to the following elements of the course. How satisfied are you with:

	Very Dissatisfied (1)	Dissatisfied (2)	Somewhat Dissatisfied (3)	Neutral (4)	Somewhat Satisfied (5)	Satisfied (6)	Very Satisfied (7)	N/A (8)
The relevance of information to your needs (1)	❍	❍	❍	❍	❍	❍	❍	❍
Presentation quality of instructor(s) (2)	❍	❍	❍	❍	❍	❍	❍	❍
Subject matter knowledge of instructor(s) (3)	❍	❍	❍	❍	❍	❍	❍	❍
The amount of information learned (4)	❍	❍	❍	❍	❍	❍	❍	❍
The way information was presented (5)	❍	❍	❍	❍	❍	❍	❍	❍
The overall quality of the course (6)	❍	❍	❍	❍	❍	❍	❍	❍

Figure 18. (Continued)

Howling Cow videos (7)	❍	❍	❍	❍	❍	❍	❍	❍
Lecture videos (8)	❍	❍	❍	❍	❍	❍	❍	❍
Interactive lesson modules (attendance) (9)	❍	❍	❍	❍	❍	❍	❍	❍
Moodle workshop activities (12)	❍	❍	❍	❍	❍	❍	❍	❍
Discussion forums (10)	❍	❍	❍	❍	❍	❍	❍	❍
Quizzes (11)	❍	❍	❍	❍	❍	❍	❍	❍

Q6 Would you recommend this program to others?

❍ No (1)

❍ Maybe (2)

❍ Yes (3)

Q7 Why or why not?

Figure 18. (Continued)

Q49 What was your motivation for taking this course?
- ❍ Required by degree audit (1)
- ❍ Required by current employer (8)
- ❍ To advance career opportunities (2)
- ❍ To learn more about food safety (4)
- ❍ Other (please specify) (7) _____

Q48 What was your preferred way to contact instructors?
- ❍ Face-to-face (1)
- ❍ Email (2)
- ❍ Office hours (3)
- ❍ Forum activities (4)
- ❍ N/A (6)

Q47 Please rank the order of instruction on what you thought to be the most helpful in understanding HACCP. 1 being the best and 7 being the worst
_____ The discussion forums (1)
_____ The game-based learning activity (2)
_____ The interactive lesson modules (attendance) (3)
_____ The quizzes (4)
_____ Lecture videos (6)
_____ The howling cow videos (7)
_____ Moodle workshop activities (8)

Q63 To what extent do you believe that:

	Disagree strongly (1)	Somewhat disagree (2)	Disagree (3)	Neither Agree or Disagree (4)	Somewhat agree (5)	Agree (6)	Strongly Agree (7)
The Howling Cow video case studies helped apply HACCP concepts in a real world situations (2)	❍	❍	❍	❍	❍	❍	❍
Click to write Statement 5 (7)	❍	❍	❍	❍	❍	❍	❍

Figure 18. (Continued)

The Howling Cow Ice Cream Makers video helped you understand what a typical work day is like in a food processing facility (4)	❍	❍	❍	❍	❍	❍	❍
The Howling Cow Processing facilities increased your understanding of how dairy products are processed (5)	❍	❍	❍	❍	❍	❍	❍
The Howling Cow documentary interview increased your understanding of regulations regarding HACCP and dairy products (6)	❍	❍	❍	❍	❍	❍	❍

Q60 What do you like the most about this course?

Q61 What do you like the least about this course?

Q62 What do you suggest to improve this course?

Q58 Thank you for taking your time to complete this survey. Please upload the phrase "WOLF" for the survey assignment