12-2008

Novel Method for Recovery of *Escherichia coli* O157:H7 from Beef Surfaces

Matthew Adam Becher

University of Tennessee - Knoxville

To the Graduate Council:

I am submitting herewith a thesis written by Matthew Adam Becher entitled "Novel Method for Recovery of *Escherichia coli* O157:H7 from Beef Surfaces." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Food Science and Technology.

David A. Golden, Major Professor

We have read this thesis and recommend its acceptance:

P. Michael Davidson, Doris H. D'Souza, Arnold M. Saxton

Accepted for the Council:
Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

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

To the Graduate Council:

I am submitting herewith a thesis written by Matthew Adam Becher entitled "Novel Method for Recovery of *Escherichia coli* O157:H7 from Beef Surfaces." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Food Science and Technology.

_____
David A. Golden, Major Professor

We have read this thesis and
recommend its acceptance:

_____
P. Michael Davidson

_____
Doris H. D'Souza

_____
Arnold M. Saxton

Accepted for the Council:

_____
Carolyn R. Hodges, Vice Provost and
Dean of the Graduate School

(Original signatures are on file with official student records)

Novel Method for Recovery of *Escherichia coli* O157:H7 from Beef Surfaces

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

Matthew Adam Becher
December 2008

ACKNOWLEDGEMENTS

My highest praise goes out to Dr. David Golden, my major professor, for continually broadening my educational experience allowing me to complete my M.S. degree. He not only provided me with a great mentor but also a superb friend that kept my educational experience not only beneficial but enjoyable. I also would like to reach a special thanks out to the entire Food Science Department. The help and encouragement that I received from Faculty, staff and fellow student body showed how special of a department this one was. The knowledge and friendships I gained are irreplaceable.

I would like to extend my appreciation to Microbial-Vac Systems®, Inc., in specific, Dr. Bruce Bradley, Jared Maughan and Kevin Church. They provided me with the opportunity to excel my learning process by working with innovative technology they developed.

I finally would like to show my highest thanks to my wife, Alicia Becher, and my family for all of the poignant help they provided through this arduous time. No matter the difficulties I faced and the emotional side effects that followed, they were there to keep me up-beat and excelling at the task at hand. I cannot thank them enough for the work they did to help me, which directly impacted my finished product.

ABSTRACT

A study was conducted to compare the efficiency of detection of *Escherichia coli* O157:H7 EDL 933 on external beef trim by commercial recovery systems based upon of the Microbial-Vac System (M-Vac) and excision sampling. *Escherichia coli* O157:H7 EDL 933 was cultured in tryptic soy broth for 24 hr at 37°C and transferred for three consecutive days before diluting and spot inoculating onto a 684 cm^2 piece of beef trim (10 CFU/684 cm^2). Beef trim was sampled with the M-Vac by passing the sampling head over the entire surface area in a vertical sampling pattern. The sampling head sprayed sterile buffer over the beef surface and instantly vacuumed the contents into a sample collection bottle. For the excision method, samples were cut from the beef trim using a coring knife. Sixty cores (3.8 cm diameter) were used per sample with one core directly inoculated by the *E. coli* O157:H7. Samples (M-Vac collection fluid and 60 excised cores) were held overnight at 4°C to simulate processing conditions, enriched in mEHEC broth at 42°C for up to 18 h, and confirmed positive using the BioControl Assurance Genetic Detection System (polymerase chain reaction based).

Of 75 inoculated beef trim surfaces, 96 (± 3.86) and 76% (± 3.86) of samples tested positive for *E. coli* O157:H7 by the M-Vac and excision methods, respectively. The ability of the M-Vac to detect *E. coli* O157:H7 significantly

better ($P<0.05$) than excision, combined with the nondestructive nature of the M-Vac demonstrates that it is a suitable choice for sampling beef surfaces.

Table of Contents

LIST OF TABLES

Table **Page**

LIST OF FIGURES

Figure **Page**

CHAPTER I

Literature Review

Hazard Analysis and Critical Control Points

The microbiological safety of beef related products remains an area of great concern among food processors regulatory authorities. To combat the occurrence of physical, chemical or biological hazards in a meat production facility, a scientific process to control these vulnerabilities was mandated in 1996 by the Food Safety and Inspection Service (USDA, 1996). This process is known as the Hazard Analysis Critical Control Point (HACCP) system. The HACCP system for meat inspection is based on inspection of products and equipment for visible contamination and then subjective testing to determine the effects created by individual operations within the production process itself (Brown et al., 2000). To properly identify Critical Control Points (CCPs) within a production process, it is suggested to base these locations on microbiological data that allow the estimation of indicator organisms at multiple stages throughout the process (Gill et al., 2003).

In meat production, specifically beef, the HACCP system can be based on interventions, a non-intervention system or combination of both techniques. The use of intervention strategies allows a consistent reduction in bacterial

contamination with minimal manual input. Examples of decontamination steps are spraying/washing of carcasses by hot water or steam vacuuming at 85°C. The bactericidal effect of these techniques is mainly thermal, although an additional physical effect by removal of the bacteria may occur also (Bolton et al., 2001). There are inherent negatives that accompany these operations such as the use of 85 to 90 gallons of water per second with possibilities of discoloration of the carcass surfaces (Bolton et al., 2001). Also, in some areas the carcass surface may only reach temperatures of 34-49°C and fecal contaminates may just be redistributed rather than removed (Bolton et al., 2001).

The use of organic acid sprays, such as lactic or acetic acid, is also an intervention method. This is widely used in the US, but prohibited in many other countries. Considered more a Good Manufacturing Practice (GMP) rather than a CCP, organic acid sprays are not applied to carcasses with an open wound or leaking abscess and may cause discolorations (Gill et al., 1999). Also it is not clear if significant lethal effect occurs on its own and use may cause respiratory or skin/ eye irritation to operators (Bolton et al., 2001). This can be used in combination with hot water washing to create a synergistic effect against microorganisms. These interventions are done at different stages during the slaughter process such as first/second legging, hide removal, pre-evisceration and/or trimming (Pearce et al., 2004).

The introduction of ‘zero tolerance’ with organisms such as *E. coli* O157:H7 has created a greater reliance on trimming as an intervention step. If there is any visible contamination such as feces, ingesta or milk (in the case of

cows) it is trimmed off of the carcass surface (Bolton et al., 2001). A significant decrease in carcass contamination occurs as long as knives and hooks are properly sterilized.

Chilling does not generally get recognized as an intervention step due to its use for holding. It does however reduce the number of reported carcasses contaminated with pathogens. Borch et al. (2002) reported that confirmed *E. coli* O157:H7 was reduced from 32 to 7% and presumptive *E. coli* O157:H7 reduced from 42 to 22% on beef carcasses. The exact parameters (air temperature, relative humidity, air speed and carcass spacing) that create such a reduction in microbiological counts have yet to be determined and established, but any such parameters are seen as positive effects (Bolton et al., 2001).

Many processing plants, such as European, see intervention HACCP strategies as a means to conceal or compensate for poor hygiene standards within the process (Bolton et al., 2001). It is seen that achieving proper hygiene measures throughout the meat processing will overcome the threat of *E. coli* and avoid detrimental effects as discoloring of the meat carcass that intervention methods provide. These non-intervention systems contain four CCPs: de-hiding; evisceration; removal of the spinal cord; and chilling. The goal is to keep utensils sterile and through proper techniques and monitoring preventing the cross-contamination of fecal and other possibly pathogen rich material (Bolton et al., 2001). If properly done at these CCPs, Bolton et al. (2001) reports carcass contamination levels decreasing approximately from 8 to 1.5% with an affiliated decrease of aerobic plate count (APC) of 99.8%.

Pathogenic microorganisms on raw beef

Foodborne pathogens have been demonstrated to be associated with red meat and meat products since the time of Pasteur. *Salmonella*, *Staphylococcus aureus*, *Bacillus cereus* and *Clostridium botulinum* are pathogens that have been shown to be associated with meats since the early 1900s. More recently the emergence of *Campylobacter* spp., *Listeria monocytogenes* and *Escherichia coli* O157:H7 have been shown to be associated with red meat products and have been involved with a number of foodborne illness outbreaks and recalls (Borch and Arinder, 2002).

The first identification of a microorganism that could cause an epidemic diarrheal disease occurred in 1888. A German hygienist and bacteriologist, August Anton Hieronymus Gärtner, revealed a pathogen that would be later described as *Salmonella enteritidis,* causing such gastroenteritis in rodents and humans (Merriam-Webster, 2008). In 1953, raw meat was implicated as the origin of a *Salmonella Typhimurium* outbreak in Sweden, causing 8845 reported cases leading to 90 deaths (Borch and Arinder, 2002). It continues to be a problem today with antibiotic resistant strains emerging in the 1990s and causing difficulties in eradicating from infected farms (Borch and Arinder, 2002).

One of the earliest foodborne outbreaks reported occurred in 1906. The symptoms described were similar to the attributes of the pathogen *Bacillus cereus* found in meat products (Borch and Arinder, 2002). B. *cereus* is of concern because of psychotropic strains that can withstand heat and refrigerated

temperatures. These strains are mainly found in dairy products but have occurred in broth mediums used with meat products (Reid et al., 2002).

Many of these pathogens are found naturally in the gastrointestinal tracts of cattle or in the surrounding environment (soil, water, etc) where cross-contamination can easily occur. This leads to high prevalence rates in cattle such as with *Salmonella* and *Campylobacter* spp., which have rates of 5.5% and 5.0-53.0%, respectively (Reid et al., 2002). *C. botulinum* is also widespread in such environments with non-proteolytic types present in 73% of cattle feces. The main foods associated with *C. botulinum* are canned, especially home-preserved, creating an ideal anaerobic environment. In the early nineteenth century, it is thought to be responsible for deaths associated with home-cured hams and sausages (Hauschild, 1989).

The severity of verotoxigenic *E. coli* (VTEC) along with its use of ruminants as a reservoir to survive increases its ruthlessness in meat processing facilities (Borch and Arinder, 2002). The VTEC group, which consists of *E. coli* O157:H7 and other serotypes, creates a similar if not identical toxin to that of *Shigella dysenteriae*. This verotoxin can attack the colon of an infected individual causing the initiation of abdominal disorders. When the *eae* gene is present, attachment and effacement of the cell occurs causing bloody diarrhea. If left untreated, hemolytic ureic syndrome (HUS) can occur, with a possible result of renal failure (Elder et al., 200). *E. coli* O157:H7 has a typical prevalence rates in cattle range from 1.0 to 27.8% and even up to 68% in heifers (Reid et al., 2002). Seasonal variation creates influences on the pathogens presence, but Elder et al.

(2000) report that typically a 10.7% incidence rate of *E. coli* O157:H7 can be found on cattle hides in the USA. Several of the VTEC serotypes that are shed in cattle feces have been traced to human illness cases (Reid et al., 2002).

It is estimated that annually 10,000 cases attributed to *E. coli* O157:H7 occur in the United States. In 1993, between the months of March and August, an epidemic of *E. coli* O157:H7 infected a restaurant chain in Northwestern U.S. The outbreak was attributed to cross-contamination, most likely by raw beef (Jackson et al., 2000). More recently, a multi-state outbreak of *E. coli* O157:H7 was attributed to a manufacturer of frozen beef patties. Between July and September of 2007, 40 documented cases were linked to the beef patties resulting in a recall of 21.7 million pounds of frozen ground beef (Centers for Disease Control and Prevention, 2007).

Modes of contamination with *Escherichia coli* O157:H7

Beginning in 1982, *Escherichia coli* O157:H7 was recognized as the source of epidemic bloody diarrhea from foodborne sources and outbreaks are continually seen today (Jackson et al., 2000). Of the documented cases, over half of them have been attributed or linked to foods originating from cattle. Cattle are a primary reservoir of *E. coli* because the microorganism exists naturally in the gut of ruminant animals such as cattle (Elder et al., 2000). The cattle then become a carrier for the bacteria that is harbored in the processing environment

(Borch and Arinder, 2002). The bacterium that originates from the animal, either via feces or from the hide, subsequently is a possible source of cross-contamination during processing.

Initial cross-contamination points occur at pre-evisceration stages in which feces to hide or hide to hide contamination occurs. It is suspected that during holding and transport of cattle, the close quarters create greater chance of hide cross-contamination occurring (Elder et al., 2000). In a study conducted by Elder et al. (2000), almost half (45.5%) of the tested carcasses had *E. coli* O157:H7 recovered. It is to be noted that the study was conducted during the peak time for *E. coli* O157:H7 shedding for North American cattle, late summer and early fall. Contamination to the hide can further be seen in feedlots in which feces carrying the bacteria appeared on the hides of cattle and ultimately contaminated carcasses in the processing plant (Aslam et al., 2003).

The association of fecal matter and *E. coli* creates an influence in how the hide removal occurs during processing. Tag (mud, bedding or manure) is carried on the hide and its mixture of soil and feces (both sources of *E. coli*) can contaminate the hide with upwards of 9.0 $\log_{10}$ bacteria per cm^2 (Van Donkersgoed et al., 1997). If not properly detached prior or during hide removal, contamination could occur further down the processing line. The removal, however, provides a new problem as labor costs increase and production speed decreases by 10 to 12% increasing those costs also (Van Donkersgoed et al., 1997).

It is typically presumed that if equipment is routinely cleaned to standards that the *E. coli* found on meat is deposited from dressing the carcass and that few to no additional *E. coli* is added during breaking of the carcass (Gill et al., 2001). Some recent studies have revealed that *E. coli* numbers actually increased for some meat cuts at the end of the carcass breaking process rather then prior to any evisceration stages. It has even been seen in some lots that when no fecal or hide tests were seen as positive that carcass samples post processing were positive (Elder et al., 2000). These results suggest cross-contamination or recontamination occurring during the breaking and splitting of the carcass. McEvoy et al. (2004) observed logarithmic increases in *E. coli* numbers during evisceration and almost a full order of magnitude increase during splitting. One site that saw this increase was at the cranial back which is never in direct contact with the visceral contents. It was seen to contact elevated sides on the evisceration table and the splitting stand as it moved downward past the site during processing. These sites are in contact with visceral contents and possible fecal matter showing a possible route of contamination. Further studies by Gill et al. (2001) showed similar numbers of recovered *E. coli* and coliforms on equipment surfaces as meat carcasses after the passage of the product through the processing line. These studies indicate possible contamination during processing by sub-standard cleaning of processing equipment.

Attachment

In order to survive and grow, microorganisms show attraction to surfaces that contain necessary nutrients. A food system, such as a meat surface, provides such necessary qualities and is sought out by microorganisms. Once a microorganism is deposited upon a surface, they quickly attach, begin to grow and actively create a colony of cells (Kumar and Anand, 1998). This causes severe problems in meat products as spoilage and pathogenic bacteria are of major concern. The understanding of what influences bacteria's attachment and how these strong bonds to the meat matrix occur is thereby essential for determining prevention and removal methods.

In general, the process of bacterial attachment is seen as a two stage process. This process may be active or passive with strong dependence on the motility of the bacterium or the transportation of planktonic (free floating) cells by diffusion, gravity or fluid forces from the surrounding environment (Kumar and Anand, 1998). The first phase is seen as a reversible stage in which mainly long range physio-chemical forces occur (Kumar and Anand, 1998, Benito et al., 1997). These forces include van der Walls attraction forces, electrostatic forces and hydrophobic interactions (Kumar and Anand, 1998). As these weak electrostatic interactions occur, the bacteria still show motion and can easily be removed through fluid shear forces such as rinsing (Kumar and Anand, 1998, Warringer et al., 2001).

Bacterial hydrophobicity refers to the tendency of the microbial cell to have a stronger affinity toward similar cells or molecules on the target surface rather then water molecules (Rivas et al., 2006). It is highly debated whether or not a positive relationship between attachment and hydrophobicity truly exists. Different methods for determination of bacterial adherence relationship to hydrophobic interactions exist, such as bacterial adherence to hydrocarbons (BATH), hydrophobic interaction chromatography (HIC) and the salt aggregation test (Kumar and Anand, 1998). Benito et al. (1997) concluded a significant correlation in the relationship by use of BATH while Dickson and Koohmaraie (1989) found no correlation between the two in use of the same test. Instead Dickson and Koohmaraie (1989) determined that the surface charge of the bacteria, not hydrophobicity, held the important factor in initial attachment. It is generally agreed that bacterial attachment has a stronger affinity for adipose tissue than lean tissue, but due to what forces is in high disagreement. To even further the debate, such surface properties as muscle or tissue type, age, pH and temperature of the contact surface can change and influence initial attachment (Kumar and Anand, 1998, Rivas et al., 2006).

It is also highly disputed whether surface structures, including flagella and fimbriae, are important factors in the attachment process. Past studies have shown that nonfimbriated and nonflagellated cells have attachment rates similar to those consisting of those structures while other reports indicate motile bacteria have increased rates of attachment (Dickson and Koohmaraie, 1989). It has also been shown that *E. coli* O157:H7 shows an increase in hydrophobicity when

such surface structures are expressed, indicating that the structures do increase attachment rates (Rivas et al., 2006). Furthermore, if the bacterium has motility it may increase its ability to find home in surface pores or crevices located on the substratum of the meat. During rigor these spaces are formed by the shrinkage of muscle fibers and the resulting channels provide entrapped bacteria with much protection from outside influences (Rivas et al., 2006). Overall it is most likely that the role of flagella in attachment is more dependent on the specific strain and growth conditions at the time of attachment and aid in the adhesion opposed to causing the bonding.

After initial bacterial adherence to the meat surface the second stage, irreversible adhesion, occurs through short-range forces. These are stronger interactions such as dipole-dipole, hydrogen, ionic and covalent bonding along with bridges forming between the bacterial cell and substratum by polymeric fibrils (Kumar and Anand, 1998). Often in the second stage, the bacteria will secrete extracellular polysaccharides leading to stronger attachment and formation of a complex community of cells (Benito et al., 1997). The bacterial cells then grow and divide by use of the nutrients present on the substratum of the meat surface. This present microcolony then produces additional polymer (EPS) which increases the stability and anchorage of the colony to the surface (Kumar and Anand, 1998). This stabilizing factor allows the cells to endure the fluctuations of the surrounding environment, such as heat, acid, and osmotic stresses. These protections from killing effects of these stresses make eradication of the bacterial cells even harder (Kinsella et al., 2007). Removal

must now be done by strong forces like scrubbing or scrapping which ultimately may damage the integrity of the meat surface itself (Kumar and Anand, 1998). If not removed, the bacterial cells will continually attach and grow forming a biofilm over time. A fairly slow process, but such a matrix can overtake a surface with a damaging millimeter thick layer of spoilage or pathogenic microorganisms (Kumar and Anand, 1998).

Detection methods for *E. coli*

Sponge/Swab

Since the early 1900's, how to detect and enumerate microorganisms on surfaces has been of great concern to microbiologists. As a great deal of circumstantial evidence implicated multiple use eating utensils in the spread of viral and bacterial infections, the rise of sampling techniques began. The first technique designed to assess the contamination levels of such surfaces was the swab-rinse technique designed in 1917 by Manheimer and Ybanez (Favero et al., 1968). This initial use of the swab-rinse technique used a moistened cotton swab that was rubbed on the targeted surface. The swab head was then aseptically placed into a test tube containing a sterile diluent, mixed vigorously and the resulting fluid was plated out onto the appropriate culture media for enumeration.

The swab technique has evolved over time to create more sensitivity toward the microorganisms targeted and the surface being sampled. As it was

initially used for the smaller area food contact surfaces, now with larger surface areas being sampled, the cotton swab has been replaced in some instances by a larger sponge for carcass surfaces (Eblen et al., 2005). The United States Department of Agriculture (USDA) Food Safety and Inspection Service (FSIS) have created guidelines on the proper technique for swabbing cattle carcass surfaces. A sterile sponge is first hydrated with 10 mL of sterile diluent which is used to swab three areas as defined by a sterile template. Each area is recommended to be a minimum of 10 by 10 cm and swabbed in 10 vertical strokes followed by 10 horizontal strokes with the same sponge. It is advised to use the reversed side for the final site of the three designated for sampling, but not required. The three designated areas should be on the outer trim side of the flank, brisket and rump of the cattle carcass. The sponge is then aseptically placed into a sterile stomacher bag where an additional 15 mL of sterile diluent is added, making a total of 25 mL. The combination of the sponge and the diluent is then stomached in the original bag for 2 min and analyzed according to FSIS regulations (USDA, Food Safety and Inspection Service, 1998).

The majority of processing plants adopt the USDA-FSIS method despite it explicitly stating that methods deemed equivalent are acceptable (Gill and Jones, 1998). Recommendations provided by the USDA-FSIS may actually create awkwardness when performing (such as holding a template and swabbing at hard to reach angles) and could be improved upon. The swabs are generally still accepted in the industry because they do provide the best access to such geometrically abnormal spaces (Foschino et al., 2003). Also the swab method

inherently obtains simplicity of use, familiarity and ability to be adequate in a variety of settings, despite its downsides including generally low recovery on porous surfaces (Kang et al., 2007).

A surface sampling method is deemed efficient by its ability to remove microorganisms from a surface and in turn displace those cells from the collection material (Buttner et al., 2007). The swab sampling technique has many factors that can influence this efficiency and repeatability, including sampling collection material and size, the sample processing protocol, the surface material, the properties of the target microorganism, the manual skill of the operator, the recapture technique and the analysis method (Buttner et al., 2007). For instance, Buttner et al. (2007) found that using a cotton swab opposed to a sponge generally found more efficiency in collection. Using even the same swab technique has seen greater mean aerobic plate counts (APC) for smaller areas, 25 cm^2, compared to larger areas, 100 cm^2 (Miraglia et al., 2005). However, due to the larger area the sponge was able to cover compared to the cotton swab, when results were expressed by templates collected per sample, the two were comparable. Also the greater area covered by the sponge allows less materials used and a greater chance of capturing pathogenic microorganisms that exist at low numbers, such as *E. coli* O157 or *Salmonella* (Lindblad, 2007).

As microorganisms attach to the meat carcass surface, they eventually begin to form stronger attachment bonds that need greater scrubbing or scratching to remove (Kumar and Anand, 1998). Due to this the material that the

swab/sponge is designed out of provides an essential role. The traditional material used for swab/sponge design was cotton wool, but recent studies have employed other materials such as cheesecloth, griddle screens, 3M mesh, macrofoam, rayon, polyester, cellulose acetate and calcium alginate wool. Dorsa et al. demonstrated that the more abrasive materials have higher recovery of microorganisms than cotton wool, and rates similar to the excision technique. A recent study even revealed that macrofoam swabs recovered ≥ 30% more spores then that of rayon, polyester and cotton wool (Rose et al., 2004). The material viewed under a scanning electron micrograph showed the macrofoam to be the most open structure; having what would be characterized as a traditional sponge matrix visualization (Rose et al., 2004). The use of cellulose acetate in sponges has also shown statistically significant recovery rates greater than cotton wool, but only seemingly has advantageous recovery on beef carcasses and not those of lamb or pork (Pearce and Bolton, 2005).

The ability for the sampling device to release the microorganisms from its woven matrix is highly important to a sampling technique. Swabs made from calcium alginate wool present the ability for the swab to dissolve in the presence of Ringer's or sodium hexametaphosphate solutions releasing caught microorganisms. Unfortunately, evidence shows that the material recovers lower levels of cells then that of traditional cotton wool and inhibits growth of some microorganisms (Rose et al., 2004, Favero et al., 1968). When experimenting with cotton, macrofoam, rayon and polyester, Rose et al. showed that polyester released significantly lower percentages of microorganisms then the other

materials (83.8%, $p < 0.01$). Further assistance in the detachment of the microorganism from the swabbing material is in the extraction method. Traditionally a stomacher machine or manual hand massaging is used to physically release the microorganisms into the diluent, but more recent studies have used novel technology such as vortexing and sonication. Rose et al. revealed that vortexing had significantly greater recovery than sonication and past traditional techniques. It has been further seen that use of a surfactant combined into the sterile diluent allows for the 'washing' or releasing of certain microorganisms at a higher rate then diluent itself. Traditional extraction methods, however, are still adopted by processing plants at a higher percentage because they provide a familiarity, simplicity, and are cost and time efficient (Rose et al., 2004).

The type of surface material being sampled also highly attributes to the recovery rate of the swab sampling technique. Higher concentrations of cells are typically recovered from smooth, non-porous material such as glass and metal (Buttner et al., 2007). The surface of a beef carcass provides the furthest opposite, with multiple cracks and crevices that attachment of microorganisms. Removal of cells at high concentrations from such a porous surface takes a great degree of pressure and scrubbing action from the technician of the swab/sponge. This creates a large decline in accuracy and repeatability for a non-automated method, dependent solely on the manual operation of the technician (Kang et al., 2007). Standardization of the swabbing pattern and particularly the angle and degree of pressure applied to the swab is also problematic in the reproducibility

and repeatability which can lead to variability in results from study to study (Moore and Griffith, 2002).

Excision

The number of techniques designed to enumerate microorganisms from meat surfaces has been vast over the past 100 years. Separated in two categories, destructive and non-destructive, the non-destructive techniques have shown the highest prominence of designing novel techniques. The contact method has vast arrays of techniques (agar syringes, RODAC plates, agar sausages, membrane filter blots, self-adhesive tapes, etc.) with the main advantages of no surface damage and the simplicity and quickness that it takes to perform the tasks. However, such methods are found to be inapplicable when bacterial counts are greater then 100 CFU/cm^2 because of plate overcrowding. Also counts are not representational of larger surfaces and provide little to no suitability for crevices in meats, giving them less than 1% of accuracy compared to excision and sponge techniques (Capita et al., 2004). Direct rinsing and shaking of surfaces in diluents finds removal close to stomaching and greater then sponge technique with no damage to surfaces, however, it is only suitable when dealing with substantially small meat cuts, not emblematic of an entire carcass. Excision and swabbing methods are the most accepted techniques as they are simple in use with little required amounts of specialized material and provide high reproducibility in data. Excision is deemed the most accurate while swabbing has the highest practicality (Capita et al., 2004).

The majority of relevant studies reveal that excision and blending or stomaching is the most effective carcass sampling method. The excision technique removes pieces of tissue via sterile blade or round coring knife and the removed piece is aseptically placed in a peptone saline solution for homogenization. Proper maceration provides less variable and more reliable bacteria counts and results in almost complete recovery of firmly attached bacteria (Capita et al., 2004). Ware et al. (1999) demonstrated that on a beef carcass inoculated with *E. coli* at a level of 10^6 CFU/cm^2, after 24 hr chilling excision was still able to recover 2.5 to 4 log CFU/cm^2. This was significantly greater then sponge sampling (1.7 to 2.4 log CFU/cm2; $p \leq 0.05$) however both had similar results when sampling was followed directly after inoculation. The period after inoculation during chilling may have allowed for firmer bacterial attachment, penetration or biofilm formation, thereby reducing the cell recovery and efficacy of sponge sampling but still allowing excision sampling to recover significant results (Capita et al., 2004).

The variability of recovery for sponge technique is quite high, ranging between 0.01 and 89% of what is achieved by the excision method (Pepperell et al., 2005). This difference in recovery results is related to numerous factors, such as the fore mentioned storage time before sampling. A high microbial load on the carcass surface effects recovery by increasing the ease of removal of cell colonies, thus increasing swabbing results to levels more similar to excision (Capita et al., 2004). The surface being sampled creates recovery variation depending on if its fat content, with high adipose tissue samples leading to lower

relative counts in swabbing but less variation for excision occurs (Pepperell et al., 2005). Pepperell et al. (2005) revealed that differences even exist by species-related microflora, as results were more similar to excision for swabbing when recovered from beef compared to pork. These variations are not seen in the excision method, nor do the chances of recovered cells being redistributed to the sampled surface exist as do with the swabbing technique (Pepperell et al., 2005).

In a study performed by Palumbo et al. (1999) swine carcasses were analyzed for bacterial counts (*E. coli*) after a 24 hr refrigerated storage. The excision method recovered an average of 2.35 ± 1.05 log CFU/cm^2 from the surface samples which was significantly higher then using a three-site swab method (0.27 ± 0.95 log CFU/cm^2). This is very consistent with results from other publications. Similar results were found by Mirigalia et al. (2005) as swabbing only produced a mean log CFU/cm^2 of 2.26 compared to the significantly higher ($p < 0.05$) excision mean of 3.46. Such results demonstrate that excision consistently recovers greater bacterial counts then other non-destructive methods. However, because the excision method is time consuming, requires high level of expertise, is destructive in nature and involves sampling only small limited areas, the nondestructive sponge/swab technique is presently used by most processing facilities (Capita et al., 2004).

The effectiveness of swab recovery compared to excision is highly dependent on the swab material. As excision yields the highest recovery results, and cotton wool swabbing the least effective, a variety of sponges, cloths and meshes of different degrees of abrasiveness fall in between, with some similar to

excision (Byrne et al., 2005). Pearce and Bolton (2005) performed a study comparing abrasive sponge materials to the excision method and found that with polyurethane sponge on pork and lamb carcasses similar recovery counts to excision were obtained. Other authors report similar results from use of more abrasive sponge material (Gill and Jones, 2000). Despite that, there is still no true consensus of the relative numbers that either method recovers, thereby also no conversion factor exists to better compare relative hide cleanliness between sampling techniques (Gill et al., 2001).

As it is generally unnecessary to have large sample size for enumerating total aerobes on a carcass, it is quite desirable for enumerating microbes that are vast and unevenly distributed on a carcass, such as *E. coli* O157:H7 and *Salmonella*. Data provided by Gill and Jones indicates that for such sparse microbes, increasing the surface area sampled by 10-fold will nearly double the incidence of their recovery. This makes swabbing very beneficial for detection of bacteria with low incidence and uneven distribution on the carcass. When comparing three Swedish abattoirs, Lindblad (2007) revealed that excision reported significantly less percentage of positive samples (3, 14 and 3%) at the abattoirs as did the sponge method (55, 84 and 52%). The size restrictions on the excision method only allowed for a total area of 20 cm^2 to be evaluated per sampling compared to 400 cm^2 for the sponge method. The ability for the swabbing to cover such a large area, increasing reliability for monitoring sparse pathogenic microorganisms, and being less laborious with no compromising of

the meat surface, makes it highly advantageous for the meat industry (Lindblad, 2007).

Contact and rinse methods

Nondestructive sampling techniques provide simplicity and quickness that are beneficial for the operator. A large demographic of the nondestructive methods are described as contact techniques. These methods utilize direct contact with the targeted surface and the growth media. Seen in a variety of variations (agar syringes, agar sausages, impression plates), each provide the possibility of direct microscopic examination or plating and incubation (Moore and Griffith, 2002). The direct contact of the two surfaces produces a mirror image of the distribution of bacteria on the targeted surface (Capita et al., 2004).

Contact methods have been seen to provide results closely correlated with the excision methods when *E. coli* are low in number and attached to smooth surfaces. Counts greater then 100 CFU/cm^2 cause problems for contact methods as plate overcrowding occurs. Further, on porous surfaces microbial counts are less than 1% then that of excision or blending because of the inability to recover microbes within surface crevices (Capita et al., 2004). Surface areas sampled are only as large as the contact method apparatus, leading to multiple sites sampled to yield representative data.

The rinse method is a non-destructive method that utilizes full submersion of the targeted surface within the sterile buffer and retrieval of that entire mixture. Its ability to use fluid force to detach microbes from porous surfaces have led it to

recovery levels similar to excision and up to 10 fold greater then swabbing (Capita et al., 2004). Izat et al. (1989) saw greater recover on poultry carcasses with the rinse method then sponging and hand message sampling methods. The inherent disadvantage with the rinse method is surface size. Recovering all the rinse solution is essential and is impractical with larger surfaces as carcasses and only find true validity with poultry and small meat cuts (Moore and Griffith, 2002).

M-Vac

Since the creation of the rinse/swab technique by Manheimer & Ybanez in 1917, little innovation has been seen with surface sampling techniques (Gill and Jones, 1998, Favero et al., 1968). The current techniques have collected far less attention in improvements then that of detection techniques. The contradictory problem with improvements in detection and not sampling is no matter how advanced the detection method, identification of microorganisms cannot occur if they are not recovered. Pathogenic detection is directly linked to the sampling technique (Microbial-Vac Systems, 2008).

In attempt to increase the standards of recovery for surface sampling, the Microbial-Vac (M-Vac) was created. The M-Vac consists of a vacuum pump housed in a support equipment case (SEC) along with a sterile diluent delivery system with an included high efficiency particulate air (HEPA) filtered exhaust system (Figure 1). The M-Vac provides a sampling area of approximately 1,800

cm^2 with its independently mobile sampling head (Figure 2). This allows for a higher percentage chance of discovering low level and highly dispersed pathogens (Microbial-Vac Systems, 2008). The powerful spray of sterile diluent from the sampling head allows penetration into deep cracks and crevices of porous surfaces. The simultaneous vacuuming of the solution allows for retrieval of present pathogens into the sampling collection bottle (Figure 3). The combination of diluent spray and vacuuming creates a high turbulence that helps release pathogens from the meat surface (Microbial-Vac Systems, 2008). The retrieved solution is then contained in the sampling collection bottle where direct laboratory testing can occur without any further elution steps.

The nature of the M-Vac allows for it cover a larger surface area then that of the excision method while being non-destructive. This ability gives the M-Vac a heightened sensitivity to sparse pathogens not evenly distributed about a meat surface (Gill et al., 1998). The nature of the M-Vac further creates an increased turbulence on the meat surface creating higher detachment of pathogens that are directly collected into a sample collection bottle. This not only increases pathogen recovery but also decreases lab time and supplies. The Microbial-Vac Systems®, Inc. demonstrated with low level inoculums the M-Vac's ability to collect similar levels of *E. coli* from beef surfaces (52%) compared to the excision method (54%) with significantly higher levels than the sponge technique (16%). On another porous surface (cantaloupe) the M-Vac was able to, in comparison to the excision method, recover similar levels of a high inoculum (6.9 log cfu/100

cm^2) and significantly greater levels of a low level inoculum (2.9 log cfu/100 cm^2) (Microbial-Vac Systems, 2008).

Objectives

This study was conducted to determine the extraction efficiencies of the M-Vac and excision methods for sampling external beef trim. The M-Vac will be tested to see if recovery efficiency is statistically similar or greater then that of the 'gold standard' excision method to help justify its use in the meat processing industry. A low contamination level (~10 CFU/684 cm^2) will be used for determining extraction/recovery efficiencies for both sampling methods. As a secondary aim, the potential for contamination carry-over from one sample to the next when using the same M-Vac sampling head kit for multiple samples will be evaluated.

CHAPTER II

Materials and Methods

Preparation of inoculum

Escherichia coli O157:H7 EDL 933 was used for inoculation of external (hide-side) trim meat surfaces. The test strain was cultured in tryptic soy broth (TSB; Difco Becton Dickinson Microbiology Systems; Sparks, MD) for 24 hr at 37°C. Cultures were transferred a minimum of three times at 24 hr intervals before use. The culture was serially diluted in 0.1 M phosphate buffer (PB; Becton Dickinson Microbiology Systems; Sparks, MD) to produce a population of approximately 1 CFU/mL for use as inoculum for meat samples. Target inoculum populations were confirmed by spread plating (0.1 mL) onto sorbitol MacConkey agar (SMAC; Difco Becton Dickinson Microbiology Systems; Sparks, MD) followed by incubation for 24 hr at 37°C.

Meat surface preparation and inoculation

Beef trim meat (60-lb. boxes) was obtained from a large US meat producer. Sanitized knives were used to trim meat samples to a thickness of 0.32-0.64 cm. For M-Vac samples, the beef samples were further trimmed to obtain a hide-side surface area of 684 cm^2. For excision samples, a stainless

steel sterile meet coring knife was used to create circular pieces with a diameter of 3.8 cm; one sample consisted of 60 core pieces equating to the 684 cm^2 hide-side surface area.

For M-Vac samples, 1 mL of the diluted *E. coli* O157:H7 culture suspension was inoculated in 10 aliquots at random locations over the entire 684 cm^2 hide-side surface by an individual other than the one who ultimately would conduct surface sampling. For excision samples, 1 mL of the diluted *E. coli* O157:H7 culture suspension was inoculated onto a single 3.8 cm diameter hide-side core piece. Only 1 core piece from the allotted 60 per sample was inoculated.

Storage

All beef trim meat was held overnight 4°C in plastic totes covered with plastic wrap to simulate normal processing/holding conditions of beef carcasses. Non-inoculated beef were segregated inoculated beef to prevent cross-contamination.

Sampling

For M-Vac samples, a sterile stainless steel wire grate provided a guide for the sampling head (Figure 2) on the beef trim. Beginning in the upper right-hand corner of the sample, the sampling head was passed over the entire surface area in vertical patterns, simultaneously dispensing 120 mL of surface

rinse solution (SRS) and vacuuming the rinsate fluid into the sample collection bottle (Figure 2). The 120 mL recovered sample was combined with 30 mL of 5x strength mEHEC broth and incubated at 42°C for up to 18 hr for enrichment.

In an effort to determine the potential for M-Vac system contamination carry-over, *E.* coli O157:H7 was inoculated (1 mL) into sterile Petri dishes (10 CFU/dish), and the inoculum was collected using the M-vac and 120 mL of SRS. Prior to collection, the sampling head was dipped for approximately 2 seconds into a hot water bath (85°C) two consecutive times with the vacuum in the on position. The 120 mL recovered sample was combined with 30 mL of 5x strength mEHEC broth and incubated at 42°C for up to 18 hr for enrichment.

For excision samples, an inoculated core piece (removed from 4°C storage) was combined with 59 non-inoculated core pieces to create a single sample. The core pieces were aseptically placed into a 1650 mL sterile sampling bag (VWR international, Batavia, IL) with 1.2 L of mEHEC broth (Biocontrol, Bellevue, WA) and hand massaged through the bag for 2 min. Samples were incubated at 42°C for up to 18 hr for enrichment.

Controls

Controls were made, positive and negative, for each run for both sampling procedures. Preparation, storage and sampling procedures were carried out identical to all test samples. For the inoculation step, sterile phosphate buffer was used in place of the *E. coli* O157:H7 culture suspension. For positive controls, the post sampling mEHEC enrichments were directly inoculated with 1

mL of the *E. coli* O157:H7 culture suspension. The *E. coli* O157:H7 culture suspension was held at 4°C overnight to simulate stress attributed to storage.

Detection

Enriched samples were confirmed positive using the Assurance Genetic Detection System (GDS) (BioControl; Bellevue, WA), an automated real-time polymerase chain reaction procedure. Briefly, 1 mL of enriched sample was added to 20 µL of a concentration reagent containing immunomagnetic beads. Samples were mixed on a vortex and held for 5 min to allow for IMS beads to attach to *E. coli* O157:H7 cells via specific antibody-antigen reaction. A magnetic PickPen™ (Biocontrol, Bellevue, WA) was used to transfer the magnetic beads into 35 µL of re-suspension buffer. The re-suspended sample was then added to 10 µL of DNA polymerase inside an amplification tube. Prepared amplification tubes were placed into the Assurance GDS Rotor-Gene® (Biocontrol, Bellevue, WA). A real-time polymerase chain reaction (PCR) occurs within the innovative rotary cycler presenting definitive positive or negative results after 75 minutes.

Data Analysis

The statistical model consisted of a random block design. Statistical analysis was conducted using the mixed models procedure (PROC MIXED) of SAS® 9.1 (SAS Institute Inc.; Cary, NC) (figure 4) and significance of factors set at $P<0.05$ (Saxton and Augé, 2008). Analysis of variance was used to determine

differences in the positive recovery of *E. coli* O157:H7 on meat trim surfaces. Analysis of variance ($P<0.05$) was conducted through sums of squares comparison.

CHAPTER III

Results and Discussion

Overview

The number of correctly identified positive *E. coli* O157:H7 samples by each method, excision and M-Vac, are shown in Table 1. Generally, it is agreed upon that the excision method has a higher degree of accuracy then non-destructive methods (Capita et al., 2004). The current research comparing the excision method to the novel non-destructive M-Vac method has shown significantly different results. The M-Vac method correctly identified a greater percentage (96%) of samples inoculated with 10.3 CFU/684cm^2 of *E. coli* O157:H7 (enumeration counts were 16, 4 and 12 for the first 15, 30 and final 30 samples for each method, respectively) than that of the excision technique (76%). The non-destructive nature and greater recovery rate of the M-Vac technique allows it to be a highly practical and accurate methodology in comparison to currently used procedures.

After sanitizing the sampling head in 85°C water, sterile diluent was recovered through the system to show if cross-contamination from sample to sample occurs. Results of this evaluation revealed that 1 out of 75 samples tested positive for *E. coli* O157:H7, indicating that carry-over occurred in one sample. This provides no significant data that point to a concern for cross-contamination occurring within the M-Vac system. The resulting positive sample

could be also be contributed to cross-contamination occurring during post-M-Vac sampling, such as during detection steps leading to the sample entering the BioControl Assurance GDS PCR machine.

Recovery of pathogens from surfaces

The accuracy of excision sampling is related to its direct ability to detach irreversibly adhered cells from meat surfaces after 24 hours of storage. Once cells enter the second stage of attachment, the various short-range forces created need strong forces to remove them from the substratum (Kumar and Anand, 1998). The M-Vac method creates an intense surface scrubbing force by the intensity of SRS delivered from the spray nozzle (Figure 2) across the beef surface. In addition, the force created by the vacuum adds to the ability to detach cells from the surface.

The porous nature and multiple crevices located on meat carcasses can present difficulty for non-destructive methods to retrieve cells located in those spaces. Aided by surface appendages such as fimbriae, bacterial cells are afforded protection when aggregated to the collagen fibrils found in these areas making detachment even more complex (Kinsella et al., 2007). Traditional non-destructive methods (e.g., sponge swabbing) prove inadequate to retrieve attached and embedded cells. Excision, followed by pummeling in a stomacher improves recovery, but this is a destructive and laborious method of sampling.

As such, methods that enable suitable extraction of attached and embedded cells are essential for accurate detection of target microorganisms.

Although excision and the M-Vac have the ability to retrieve attached cells, this study demonstrated that overall recovery from inoculated samples is greater using the M-Vac. A possible variation could be two-way bacterial transfer between the macerated meat sample and the surrounding solution post pummeling or stomaching. As reported in cases between a swab and meat surface by Pepperell et al. (2005), the pummeled carcass could relinquish the cells to the buffer solution, followed by a reattachment of the cells from the solution to the meat carcass. Thus, especially with low levels of inoculum (~10.3 CFU/684cm^2 in the current study) the cells could reconstitute themselves into crevices and pores of the pummeled meat creating a protective effect that would prohibit detection (Kinsella et al., 2007). Further, as in the excision procedure in this study, when multiple pieces of meat are pummeled or stomached at one time as done with cores, the inoculated side of one core could combine with another core. This would in turn place the targeted cells in between two interlocked pieces of meat, not allowing them to go into solution.

The design of the M-Vac prevents any recontamination or hidden attachment of the cells. The flow of the expulsed buffer solution will initiate detachment and collect the cells in the solution. Immediate uptake through the vacuum port sends the solution containing the target microorganism into the sample collection bottle. As long as proper protocol is followed, the solution collected in the sample collection bottle can be directly plated onto agar media or

enrichment media (as in the current study) can be added into the sample collection bottle for improved detection. From start to finish during sampling the targeted microorganisms should stay in solution without re-adherence.

Nature of the procedure

The non-destructive nature of the M-Vac adds another benefit over the excision method. As previously mentioned the excision method is destructive in nature and in turn devalues the carcass (Capita et al., 2004). This makes the excision method neither practical nor acceptable in the industry as the technique is financially undesirable. The M-Vac is itself nondestructive in nature causing no harm to the surface it covers. The rinse solution that is expelled and immediately vacuumed into the M-Vac system presents no visual harm to the surface of the carcass. It causes little to no damage to the surface of a meat carcass and would cause no degree of financial loss from physical or esthetic damage aspect.

The current study compared surface sampling techniques over the same area amount, 684 cm^2. However, in practical use the excision method typically only covers smaller areas typically ranging from 5 to 100 cm^2. The destructive nature of the procedure forces limitation on the area covered, due to carcass devaluation, along with the inevitable increase in materials, time and required expertise (Capita et al., 2004). The current study used a very large sampling area that equated to a total of 1.2 L of enrichment broth per excision sample compared to only 150 mL for each M-Vac sample. Such an increase in materials

used by the excision method is highly expensive along with highly space consuming, as approximately 10 M-Vac sample collection bottles equate to the volume taken by one excision sample in 1.6 L of enrichment broth. Further, the increased amount of mEHEC used by the M-Vac samples yields a less concentrated overall cell volume after enrichment. This puts the excision method at an immediate disadvantage.

The M-vac was able to easily cover the 684 cm^2 area, recovering 120 mL of sample. The ability to cover larger areas than the excision method results in a more reliable method for monitoring pathogenic organisms that exist in sparse and sporadic numbers upon a meat carcass (Lindblad, 2007). As the meat industry primarily targets pathogens that are sporadically located in low numbers, such as *E. coli* O157:H7 and *Salmonella*, the greater surface area coverage by the M-Vac would be beneficial for improving detection. Gill and Jones (2000) showed that when using a non-destructive method (swabbing) that covered 100 cm^2 compared to the excision method covering only 10 cm^2, a 1 log increase in recovered cells occurred. This increase in cell recovery was primarily due to the increased area sampled, not because of method as it has been shown that the swab technique is less reliable in cell recovery. Thus, as shown by the current research, since the M-Vac has the ability to detect sparse pathogenic microorganisms better than the excision method, it can be assumed that the increased sample area would produce greater than 1 log increases in microbial counts.

After sampling with the M-Vac, the solution captured within the sample collection bottle can be directly plated or have enrichment broth added for molecular detection. The excision method on the other hand requires further processing before plating or enrichment occurs. Samples would need to be taken for further homogenization (i.e., stomaching, pummeling, etc.) and possible filtration (Pepperell et al., 2005). Laboratory analyses must then be done with greater expertise and are highly time-consuming and costly.

The advantages of the M-Vac are great, with mainly its ability to recover a greater percentage of low level microbes than the excision method. The inadequately recovery and destructive nature of the excision technique makes it a less practical procedure in the detection of sparse and sporadic microorganisms. The ability of the M-Vac to detect other pathogens on beef trim surfaces compared to the excision method needs to be further studied. Also, since the study was done using only one operator of the M-Vac, further studies should be done also to determine the reproducibility from one technician to another.

Future experimental designs

The natural occurrence of microorganisms on a meat carcass can present great dilemmas. *E. coli* O157:H7 can have prevalence in cattle at a range of 1.0 to 27.8% and even up to 68% in heifers (Reid et al., 2002). Cattle are a primary reservoir for numerous coliforms and other serotypes of *E. coli* because of the

natural existence of these organisms in the gut of ruminant animals. All such microorganisms can through cross-contamination by hide-to-hide or feces-to-hide become present on the beef trimmings during processing. If meat samples are not properly taken care of before use in a study, results can be skewed. Ideally sampling should occur immediately after processing without such effects as freezing, transporting, etc. causing problems.

If background flora exists to a high degree, removing it to dismiss any microbiological competition creates difficulties. One solution could be the use of organic acid sprays, such as lactic or acetic acid, for bactericidal effects. Unfortunately such effects would affect the target microorganisms and further for some countries this technique is not used so the procedure would not be universal (Gill et al., 1999). The use of large amounts of hot water is a possibility, but it is highly expensive and may redistribute microorganisms more than removing them (Bolton et al., 2001). Both of these spray wash types of intervention have also been seen to cause discolorations and changes in the meat surface (Gill et al., 1999). Such structural changes also occur when UV rays are introduced to the meat surface to remove microorganisms, causing oxidation to compounds in the meat. These types of changes in the meat surface composition would then make the experiment not representational of practical implications in the food industry.

Further setbacks can occur by the naturally high adipose concentration existent on beef trimmings. When a sample is taken, whether by excision or M-Vac, the hydrophobic lipid particles are mixed into the buffer solution creating a

very heterogeneous system of fat particles. These lipid deposits are too large to wash through the 0.45µm direct filter membrane and prevent the proper recovery and enumeration of target microorganisms making such an enumeration method unusable.

The area of each sample presented a problem for the excision methodology. The typical area excised in practice is 5-100 cm^2 because of the increased cost, time and materials when larger areas are examined. The current study used 684 cm^2 areas that required 1.2 L of mEHEC per sample. Such a volume presented problems during homogenization and storage. These problems made post sampling procedures take a greater amount of time per sample compared to that of the nondestructive technique. Also the higher volume (1.2 L) used for the excision method compared to that of the M-Vac (175 mL total) created a less concentrated sample. If each sample recovered the maximum 10 cells, the excision method begins at a disadvantage of having near 10 times less of concentration then that of the M-Vac. The probability of recovering cells then to use for detection is inevitably less.

Experimental improvements

Main changes necessary are to avoid the interferences of the fatty nature of the beef trimmings. Since the research needs to be relatable to practical applications, the adipose tissue cannot be just removed from the carcass. Filtration steps could still be used, but the apparatus for each sample should be

disposable, causing high costs. Water baths may have bactericidal effects, but their ability to reconstitute the lipids upon the apparatus during washing causes grave effects. Avoiding the use of filtration, vortexing collected samples in order to concentrate the targeted microorganisms may be possible. After vortexing, the lipids should separate from the target microorganisms and can then be decanted off. The resulting cell pellet can then be suspended in a phosphate buffer of lesser volume to then be directly plated by pour or spread methods. The vortexing method, however, does take time and the necessity of the proper materials and expertise. The use of more rapid based detection systems such as real-time PCR can be very helpful.

The ability to distinguish between the targeted *E. coli* O157:H7 and background microflora also must occur. Barkocy-Gallagher et al. (2005) demonstrated that the use of imunomagnetic separation (IMS) beads with *E. coli* allowed for easier and increased recovery rates, as in this study. However, IMS beads are expensive and still create nonspecific binding to background flora which can make identifying positive colonies difficult (Barkocy-Gallagher et al., 2005). Others have used nalidixic acid resistant strains of *E. coli* to help in selectivity of growth during enumeration (Gill and Landers, 2004). Strains of the targeted pathogen are cultured in a nalidixic acid rich tryptic soy broth (TSB) to create a resistance to the acid. Tryptic soy agar enriched with nalidixic acid (TSAN) can be used for enumerating and the nalidixic acid can act as selective agent (Gill and Badoni, 2005). Other selective media such as ntCHROM-

O157agar can be helpful by preventing growth of background microflora with such antimicrobials as novobiocin (Brichta-Harhay et al., 2007).

CHAPTER IV

Summary

The detection efficiencies of the M-Vac and excision sampling methods on low level pathogenic microorganisms were determined. The M-Vac, with its nondestructive nature, was able to significantly detect more inoculated samples as positive then that of the 'gold standard' excision method. This can be widely attributed to the M-Vac's greater ability to detach strongly adhered cells from and within the porous beef trim surface. The nature of the excision method allows for the meat surface to stay in contact with the targeted cells and other meat surfaces which could by random physical interactions block or hide the cells from following enrichment and detection steps. Especially when multiple pieces, as with coring, are in one single buffer filled bag, the inoculated surfaces could combine with another surface hiding the cells between the two pieces, for example. The M-Vac has no step in which the cells could be hidden or redistributed to other areas; instead the cells remain in suspension in the buffer solution.

The M-Vac has the combination of abilities of greater detection and area coverage then that of the excision method. This makes it more reliable when the concern is detecting sparse and sporadically distributed microorganisms, such as *E. coli* O157:H7. Even furthering its positive attributes are its time and material

saving characteristics. Unlike the excision method, no following maceration or filtration steps are necessary for the M-Vac. This saves time and limits the level of expertise necessary to sample with the technique. Also it saves approximately 10-fold the amount of materials (e.g., buffer solutions) than that of the excision technique.

Future work does, however, need to be done comparing the M-Vac's ability to accurately detect other strains of *E. coli* and other pathogens. Future studies should look into the use of such techniques as centrifugation concentration, IMS concentration, or highly selective media to distiguish background flora and lipid deposits inherent to beef trimmings. Also, smaller sample sizes are necessary to allow for equivalent enrichment buffers to be used per sample technique.

List of References

ASLAM, M., NATTRESS, F., GREER, G., YOST, C., GILL, C. & MCMULLEN, L. (2003) Origin of contamination and genetic diversity of *Escherichia coli* in beef cattle. *Applied and Environmental Microbiology,* 69**,** 2794-2799.

BARKOCY-GALLAGHER, G. A., EDWARDS, K. K., NOU, X., BOSILEVAC, J. M., ARTHUR, T. M., SHACKELFORD, S. D. & KOOHMARAIE, M. (2005) Methods for recovering *Escherichia coli* O157:H7 from cattle fecal, hide, and carcass samples: Sensitivity and improvements. *Journal of Food Protection,* 68**,** 2264-2268.

BENITO, Y., PIN, C., MARIN, M. L., GARCIA, M. L., SELGAS, M. D. & CASAS, C. (1997) Cell surface hydrophobicity and attachment of pathogenic and spoilage bacteria to meat surfaces. *Meat Science,* 45**,** 419-425.

BOLTON, D. J., DOHERTY, A. M. & SHERIDAN, J. J. (2001) Beef HACCP: intervention and non-intervention systems. *International Journal of Food Microbiology,* 66**,** 119-129.

BORCH, E. & ARINDER, P. (2002) Bacteriological safety issues in red meat and ready-to-eat meat products, as well as control measures. *Meat Science,* 62**,** 381-390.

BRICHTA-HARHAY, D. M., ARTHUR, T. M., BOSILEVAC, J. M., GUERINI, M. N., KALCHAYANAND, N. & KOOHMARAIE, M. (2007) Enumeration of *Salmonella* and *Escherichia coli* O157:H7 in ground beef, cattle carcass, hide and faecal samples using direct plating methods. *Journal of Applied Microbiology,* 103**,** 1657-1668.

BROWN, M. H., GILL, C. O., HOLLINGSWORTH, J., NICKELSON, R., SEWARD, S., SHERIDAN, J. J., STEVENSON, T., SUMNER, J. L., THENO, D. M., USBORNE, W. R. & ZINK, D. (2000) The role of microbiological testing in systems for assuring the safety of beef. *International Journal of Food Microbiology,* 62**,** 7-16.

BUTTNER, M. P., CRUZ, P., STETZENBACH, L. D. & CRONIN, T. (2007) Evaluation of two surface sampling methods for detection of *Erwinia herbicola* on a variety of materials by culture and quantitative PCR. *Applied and Environmental Microbiology,* 73**,** 3505-3510.

BYRNE, B., DUNNE, G., LYNG, J. & BOLTON, D. J. (2005) Microbiological carcass sampling methods to achieve compliance with 2001/471/EC and new hygiene regulations. *Research in Microbiology,* 156**,** 104-106.

CAPITA, R., PRIETO, M. & ALONSO-CALLEJA, C. (2004) Sampling methods for microbiological analysis of red meat and poultry carcasses. *Journal of Food Protection,* 67**,** 1303-1308.

CENTERS FOR DISEASE CONTROL AND PREVENTION. 2007. Multistate outbreak of *E. coli* O157:H7 infections linked to Topp's brand ground beef patties. Available from: http://www.cdc.gov/ecoli/2007/october/100207.html

DICKSON, J. S. & KOOHMARAIE, M. (1989) Cell-surface charge characteristics and their relationship to bacterial attachment to meat surfaces. *Applied and Environmental Microbiology,* 55**,** 832-836.

EBLEN, D. R., LEVINE, P., ROSE, B. E., SAINI, P., MAGEAU, R. & HILL, W. E. (2005) Nationwide microbiological baseline data collected by sponge sampling during 1997 and 1998 for cattle, swine, Turkeys, and geese. *Journal of Food Protection,* 68**,** 1848-1852.

ELDER, R. O., KEEN, J. E., SIRAGUSA, G. R., BARKOCY-GALLAGHER, G. A., KOOHMARAIE, M. & LAEGREID, W. W. (2000) Correlation of enterohemorrhagic *Escherichia coli* O157 prevalence in feces, hides, and carcasses of beef cattle during processing. *Proceedings of the National Academy of Sciences of the United States of America,* 97**,** 2999-3003.

FAVERO, M. S., MCDADE, J. J., ROBERTSE.JA, HOFFMAN, R. K. & EDWARDS, R. W. (1968) Microbiological sampling of surfaces. *Journal of Applied Bacteriology,* 31**,** 336-&.

FOSCHINO, R., PICOZZI, C., CIVARDI, A., BANDINI, M. & FAROLDI, P. (2003) Comparison of surface sampling methods and cleanability assessment of stainless steel surfaces subjected or not to shot peening. *Journal of Food Engineering,* 60**,** 375-381.

GILL, C. O. & BADONI, M. (2005) Recovery of bacteria from poultry carcasses by rinsing, swabbing or excision of skin. *Food Microbiology,* 22**,** 101-107.

GILL, C. O., BADONI, M. & MCGINNES, J. C. (1999) Assessment of the adequacy of cleaning of equipment used for breaking beef carcasses. *International Journal of Food Microbiology,* 46**,** 1-8.

GILL, C. O., BRYANT, J. & LANDERS, C. (2003) Identification of critical control points for control of microbiological contamination in processes leading to

the production of ground beef at a packing plant. *Food Microbiology,* 20**,** 641-650.

GILL, C. O., DESLANDES, B., RAHN, K., HOUDE, A. & BRYANT, J. (1998) Evaluation of the hygienic performances of the processes for beef carcass dressing at 10 packing plants. *Journal of Applied Microbiology,* 84**,** 1050-1058.

GILL, C. O. & JONES, T. (1998) Comparison of methods for sampling and enumerating *Escherichia coli* on pig carcasses. *Food Microbiology,* 15**,** 617-623.

GILL, C. O. & JONES, T. (2000) Microbiological sampling of carcasses by excision or swabbing. *Journal of Food Protection,* 63**,** 167-173.

GILL, C. O. & LANDERS, C. (2004) Microbiological conditions of detained beef carcasses before and after removal of visible contamination. *Meat Science,* 66**,** 335-342.

GILL, C. O., MCGINNIS, J. C. & BRYANT, J. (2001) Contamination of beef chucks with *Escherichia coli* during carcass breaking. *Journal of Food Protection,* 64**,** 1824-1827.

HAUSCHILD, A.H.W. (1989). *Clostridium botulinum.* In M. P. Doyle (Ed.), *Foodborne Bacterial Pathogens*, 111-189.

IZAT, A. L., COLDBERG M., DRIGGERS C.D., & THOMAS R.D. (1989) Effects of sampling method and feed withdrawal period on recovery of microorganisms from poultry carcasses. *Journal of Food Protection*, 52**,** 480-483.

JACKSON, L. A., KEENE, W. E., MCANULTY, J. M., ALEXANDER, E. R., DIERMAYER, M., DAVIS, M. A., HEDBERG, K., BOASE, J., BARRETT, T. J., SAMADPOUR, M. & FLEMING, D. W. (2000) Where's the beef? The role of cross-contamination in 4 chain restaurant-associated outbreaks of *Escherichia coli* O157 : H7 in the Pacific northwest. *Archives of Internal Medicine,* 160**,** 2380-2385.

KANG, D., EIFERT, J. D., WILLIAMS, R. C. & PAO, S. (2007) Evaluation of quantitative recovery methods for *Listeria monocytogenes* applied to stainless steel. *Journal of Aoac International,* 90**,** 810-816.

KINSELLA, K. J., ROWE, T. A., BLAIR, I. S., MCDOWELL, D. A. & SHERIDAN, J. J. (2007) The influence of attachment to beef surfaces on the survival of cells of *Salmonella enterica* serovar Typhimurium DT104, at different a(w) values and at low storage temperatures. *Food Microbiology,* 24**,** 786-793.

KUMAR, C. G. & ANAND, S. K. (1998) Significance of microbial biofilms in food industry: a review. *International Journal of Food Microbiology,* 42**,** 9-27.

LINDBLAD, M. (2007) Microbiological sampling of swine carcasses: A comparison of data obtained by swabbing with medical gauze and data collected routinely by excision at Swedish abattoirs. *International Journal of Food Microbiology,* 118**,** 180-185.

MIRRIAM-WEBSTER, INC. (2008). Medical dictionary Springfield, MA. Available from: http://medical.merriam-webster.com/medical/gartner. Accessed: August 14, 2008.

MICROBIAL-VAC SYSTEMS, INC. (2008) Why M-Vac? Jerome, ID. Available from: http://www.m-vac.com/why-mvac.

MIRAGLIA, D., RANUCCI, D., D'OVIDIO, V., BRANCIARI, R. & SEVERINI, M. (2005) Comparison between carcass microbial load recovered by swabbing surfaces of different size and using the reference excision method. *Veterinary Research Communications,* 29**,** 339-341.

MOORE, G. & GRIFFITH, C. (2002) A comparison of surface sampling methods for detecting coliforms on food contact surfaces. *Food Microbiology,* 19**,** 65-73.

PEARCE, R. A. & BOLTON, D. J. (2005) Excision vs sponge swabbing - a comparison of methods for the microbiological sampling of beef, pork and lamb carcasses. *Journal of Applied Microbiology,* 98**,** 896-900.

PEARCE, R. A., BOLTON, D. J., SHERIDAN, J. J., MCDOWELL, D. A., BLAIR, I. S. & HARRINGTON, D. (2004) Studies to determine the critical control points in pork slaughter hazard analysis and critical control point systems. *International Journal of Food Microbiology,* 90**,** 331-339.

PEPPERELL, R., REID, C. A., SOLANO, S. N., HUTCHISON, M. L., WALTERS, L. D., JOHNSTON, A. M. & BUNCIC, S. (2005) Experimental comparison of excision and swabbing microbiological sampling methods for carcasses. *Journal of Food Protection,* 68**,** 2163-2168.

REID, C. A., SMALL, A., AVERY, S. M. & BUNCIC, S. (2002) Presence of food-borne pathogens on cattle hides. *Food Control,* 13**,** 411-415.

RIVAS, L., DYKES, G. A. & FEGAN, N. (2006) Attachment of Shiga toxigenic *Escherichia coli* to beef muscle and adipose tissue. *Journal of Food Protection,* 69**,** 999-1006.

ROSE, L., JENSEN, B., PETERSON, A., BANERJEE, S. N. & ARDUINO, M. J. (2004) Swab materials and *Bacilius anthracis* spore recovery from nonporous surfaces. *Emerging Infectious Diseases,* 10**,** 1023-1029.

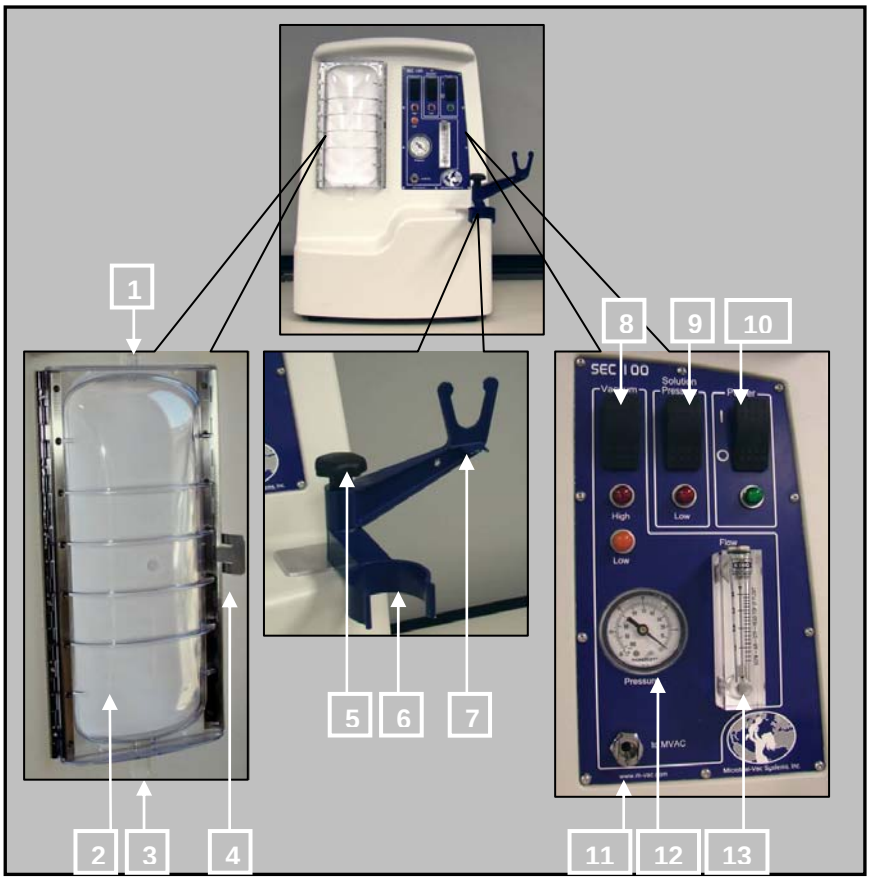
SAXTON, A.M. & AUGÉ, B. (2008) Stats Design and Analysis Web Guide. Available from: http://dawg.utk.edu/.

U.S. DEPARTMENT OF AGRICULTURE. 1996. Pathogen reduction; hazard analysis and critical control point (HACCP) system; final rule. Federal Registry. 61:38805-38989.

VAN DONKERSGOED, J., JERICHO, K. W. F., GROGAN, H. & THORLAKSON, B. (1997) Preslaughter hide status of cattle and the microbiology of carcasses. *Journal of Food Protection,* 60**,** 1502-1508.

WARRINGER, K., EVELEIGH, K., GOODMAN, J., BETTS, G., GONZALES, M. & WAITES, W. M. (2001) Attachment of bacteria to beef from steam-pasteurized carcasses. *Journal of Food Protection,* 64**,** 493-497.

Appendix



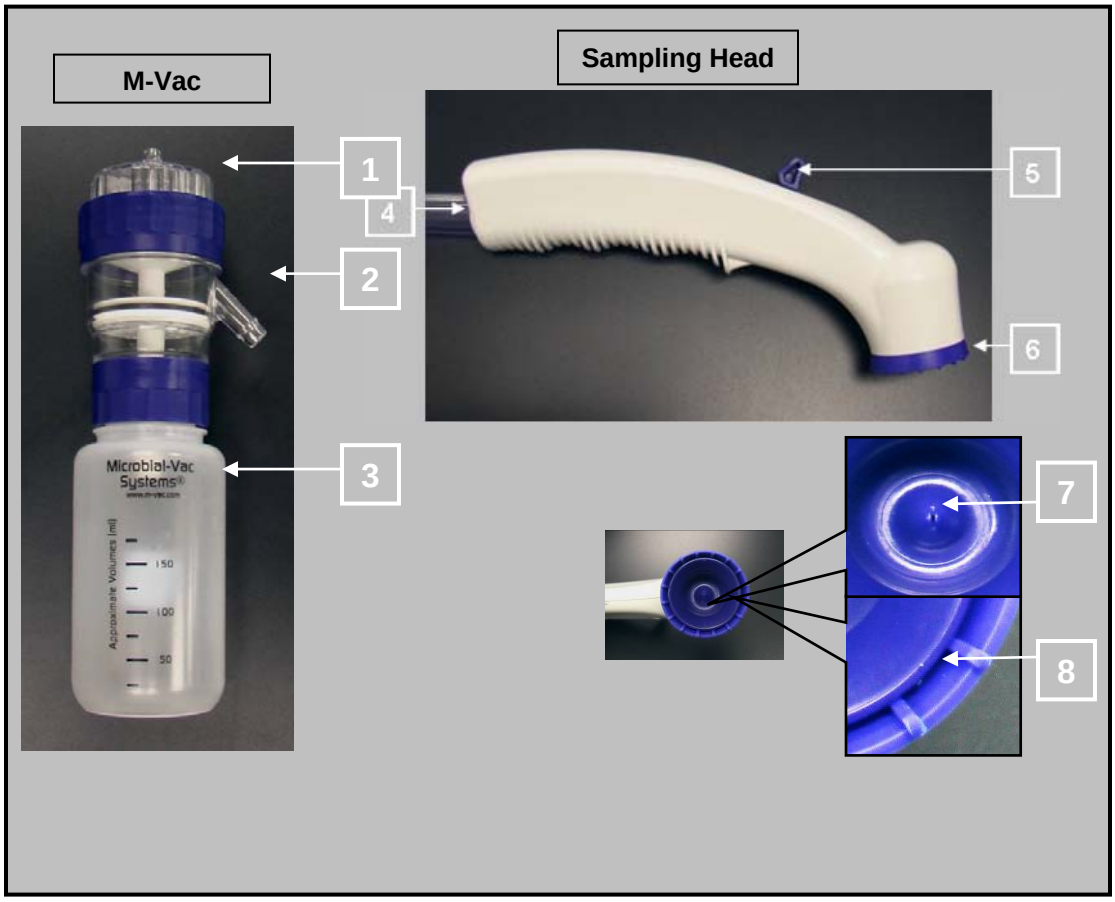
1	Hook for hanging SRS bag
2	Pressure chamber door
3	Notch for SRS bag port
4	Guide for closing pressure chamber door

5	Tension knob
6	M-Vac support clip/arm
7	Sampling head support clip/arm

8	Vacuum Power Switch
9	SRS Pressurization Switch
10	Main System Power Switch
11	Vacuum Tubing Quick Connect Port
12	Vacuum Pressure Gauge
13	Airflow Meter (Rotameter)

(Source: Microbial Vac Systems, Inc., with permission)

Figure 1. M-Vac support equipment case (SEC)

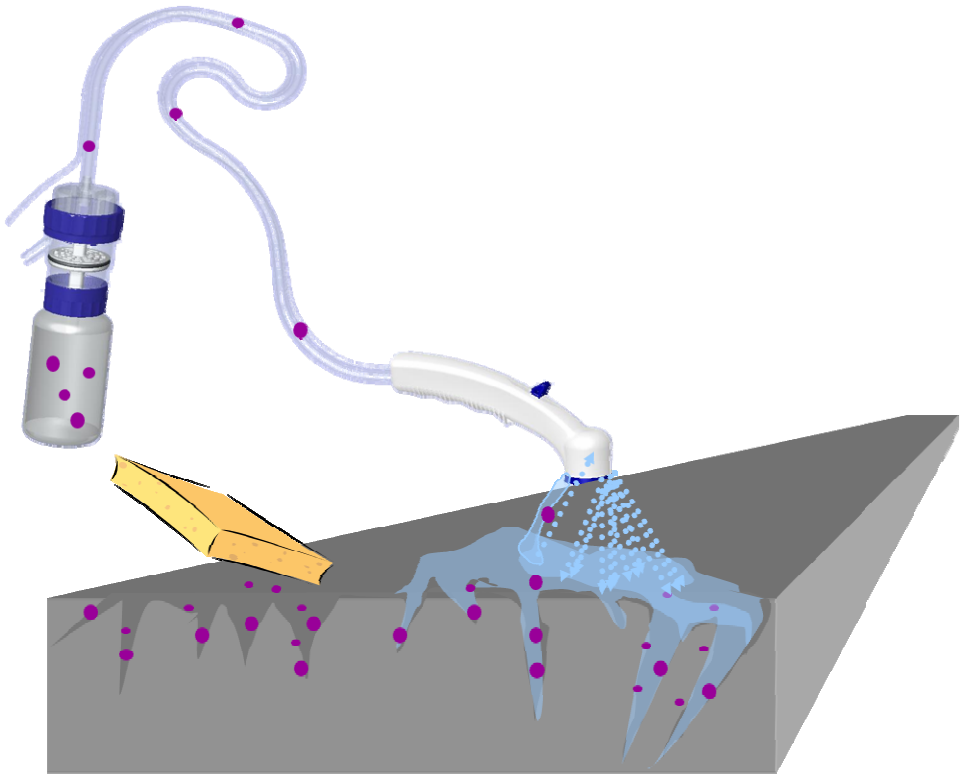


1 Lid	4 Tubing Port
2 Hose Barb for Vacuum Line from SEC	5 Switch for SRS Flow Control
3 Sample Collection Bottle	6 Flexible Surface Contact Ring
	7 SRS Spray Nozzle
	8 Vacuum Port

(Source: Microbial Vac Systems, Inc., with permission)

Figure 2 M-Vac sample collection

The M-Vac sprays sterile solution across surface to penetrate cracks and crevices while simultaneously vacuuming solution plus surface pathogens into sterile collection bottle. This turbulence greatly increases ability to extract pathogens.



(Source: Microbial Vac Systems, Inc., with permission)

Figure 3. How the M-Vac works

SAS Program

E. coli O157:H7 recovery accuracy by excision and M-Vac methodologies (Saxton and Augé, 2008)

```
proc import datafile='C:\MvacExruns.xls' out=one replace;
run;
%include'C:\DandA.sas';
%mmaov(one, positive, class=method run, fixed=method, random=run);
run;
```

Table 2. Number of correctly identified positive samples by the Excision and M-Vac methods[1]

Sampling method	Number of samples taken	Number of positive samples	Percentage of recovered positive samples[2]	Standard error
Excision	75	57	76 B	± 3.86
M-Vac	75	72	96 A	± 3.86

[1] Beef trim samples were inoculated with an average of 10.3 CFU/684cm^2 per sample

[2] Percentages of positive samples with different letters differ significantly ($P < 0.05$).

Vita

Matt Becher was born in Urbana, Illinois on October 26, 1981 to parents Michael and Margaret Becher. Matt grew up sharing time between Dallas, Texas, Goshen, Kentucky and Pickerington, Ohio. Matt spent time growing up with his younger sister Katherine Becher and graduated from Pickerington High School in 2000. He then moved to Oxford, OH to attend Miami University where he received a B.A. degree in Psychology. He then traveled to Knoxville, TN in August of 2006 to begin work in the M.S. program in Food Science and Technology focusing in Food Microbiology and graduated from this program in the summer of 2008. Matt has continued his focus in food science by pursuing a career in the field.